The Journal of Comparative Pathology and Therapeutics

Vol. 57. No. 1. JANUARY, 1947. Price 8 shillings and sixpence

1. The columns of the Journal are open for contributions recording original research in veterinary science.

2. The Editorial Board accepts papers bearing on the above subject on the understanding that they are not being offered elsewhere for publication. It reserves the right to reject any papers submitted to it for publication.

3. It is essential that all articles should be concise. Any necessary allusions to matter already published should be brief and limited strictly to those relevant to the satisfactory exposition of the authors' own work.

4. Citation of literature in the text should be accompanied by the year of publication. Authors' names (followed by year, publication, etc.) should be arranged alphabetically in a list at the end of the paper.

5. Papers for publication, exchange journals, etc., should be sent to Prof. T. J. Bosworth, "The Beeches," Streatley, Berks, or to any member of the Editorial Board.

6. Correspondence concerning business matters should be addressed to the Publishers, Messrs. H. R. Grubb, Limited, Poplar Walk, Croydon, Surrey.

7. The net price of each volume of four numbers is £1 10s., paid in advance to the publishers. Single numbers may be obtained at a price of 8s. 6d. each.

Additional copies and reprints ordered before the issue is printed may be obtained from the publishers.

CONTENTS

PAGE

The Immunological Relationships of Strains of Contagious Pustular Dermatitis Virus. By E. S. Horgan and Mansour Ali Haseeb 1

The Relationship between the Virus of Contagious Pustular Dermatitis and a Virus Isolated from Sheep Papillomatosis by Selbie. By S. E. Horgan and Mansour Ali Haseeb 8

The Prophylactic Use of Phenanthridinium 1553 against Infection with *T. Congolense* and *T. Vivax*. By H. A. Crawshaw 13

Studies on *Corynebacterium Renale*. III: Penicillin Treatment of Experimentally Infected Mice. By R. Lovell and E. Cotchin 20

The Ascorbic Acid Content of the Blood Serum of Farm Animals. By M. M. Sheahan 28

Sex Hormones in the Urine of Pregnant Cattle. By L. B. O'Moore. 36

A *Salmonella* Strain Isolated from Camels in Palestine. By L. Olitzki and V. Ellenbogen 47

Trace Element Deficiency in Livestock in the Hebrides. By G. F. Boddie 52

Leptospirosis in Cattle in Palestine. By S. Freund 62

Ornithosis (Psittacosis) in a Pigeon Flock. By D. L. Hughes. ... 67

Salmonella Typhi Murium in Penguins. By T. A. Cockburn. ... 77

J. Comp. Path. 1947. Vol. 57.

THE IMMUNOLOGICAL RELATIONSHIPS OF STRAINS OF CONTAGIOUS PUSTULAR DERMATITIS VIRUS

By

E. S. Horgan and Mansour Ali Haseeb,

Stack Medical Research Laboratories, Khartoum

INTRODUCTION

The difficulty of distinguishing goat pox from contagious pustular dermatitis on the basis of clinical symptoms alone has led to considerable confusion in regard to these two diseases in countries where both exist. This confusion has not been helped by the bewildering variety of clinical terms used to designate the latter disease.

The name contagious pustular dermatitis* was chosen by Glover (1928) as the most suitable of the many then in use and is now generally accepted in English literature. A series of cross immunity tests carried out in Khartoum (Bennett, Horgan and Haseeb, 1944) between the viruses of goat pox and C.P.D. clearly indicated that they were closely related but that goat pox possessed at least one antigenic factor which was lacking in C.P.D. In these tests only a single strain of C.P.D. virus (originally isolated by Glover in England in 1927) was used, as in an extensive series of cross immunity tests with ovine strains from England, France, and California, Glover found that in all probability they conformed to a single type. His results, however, with caprine strains from Tanganyika and Cyprus were less clear cut. The Tanganyika strain appeared to be identical with the English ovine strains, but in his own words: "The experiments with a caprine virus from Cyprus whilst affording some indication that it was immunologically related to English ovine strains have not been entirely conclusive." (Glover, 1932-33.)

It was thought that a re-examination of the matter using the standard methods evolved in the previous work in Khartoum (*op. cit.*) might prove of some interest and in the early months of 1946 the arrival of infective material from Cyprus, kindly sent by Dr. R. J. Rowe, Chief Veterinary Officer, afforded an opportunity of initiating a series of cross immunity tests.

EXPERIMENTAL TECHNIQUE

Strains used. English U.F. isolated in England in 1927 and supplied as dried material.

English No. 2. Dried scabs from an outbreak in 1946 investigated by Mr. Blakemore, of the Institute of Animal Pathology, Cambridge, who has stated that the outbreak was typical and fairly acute, but that very little work had been done with the strain, and that it had not been checked up by cross immunity tests against the standard virus.

* For the sake of brevity the initials C.P.D. will be used in this paper to designate contagious pustular dermatitis.

Cyprus No. 1. Sheep from a farm at Margo, Nicosia. Moist scabs were removed from the lips of lambs affected severely with C.P.D. and dried over silica gel for eight to ten days.

Cyprus No. 2. Goat from Paphos Forest. Dried scabs were taken from the lips of an otherwise healthy goat. The lesions resembled those of C.P.D.

Cyprus No. 3. Sheep from Larnaca. Dried scabs were removed from the lips of an adult sheep which was recovering from an attack of "Aphthous Stomatitis," a non-fatal condition seen from time to time, characterised by extensive ulceration of the surface of the tongue, dental pad and gums and accompanied by generalised reddening of the buccal mucosa and frothy salivation. Dr. Rowe thought it unlikely that C.P.D. virus would be present in these scabs.

Animals inoculated. All inoculations were made in sheep. The technique of preparaion of the virus suspension, etherisation, and inoculation by scarification on to the shaved belly has already been described in detail (Bennett, Horgan and Haseeb, 1944). The incubation periods in all animals, irrespective of the strain of virus, were constant at three to four days and the appearance and development of the lesions were of the typical vesico-pustular type as described in detail by Glover (1932-33).

For subsequent passages material from some of the vesicular eruption was gently scraped on the fifth day, ground up with 50 per cent. glycerol-water and stored at refrigerator temperature (4° C.). For stock purposes a small portion was frozen and desiccated *in vacuo* and stored *in vacuo* at 4° C.

RESULTS

The results of the inoculation of the experimental animals are given in Table I. They are presented for convenience in a single table, although the experiments were carried out in three series, *viz.*, 1st series, 1 to 6; 2nd series, 7 to 14; and 3rd series, 15 and 16. It may be noted that in Nos. 1 to 6 immunisation was effected with the naturally infected materials from Cyprus; whilst Nos. 7 to 12 were immunised with material from first sheep passage (in the case of each strain). Nos. 13 and 14 received material from the first sheep passage of the English U.F., No. 15 was infected with the natural material sent by Mr. Blakemore, and No. 16 with material from No. 15. The third series was necessary as the material from England (Strain 2) had not arrived in time for the second series. The same materials in the case of each strain were used throughout for testing immunity.

No control was deemed necessary for Nos. 1 to 6 as all inocula had been freshly prepared. Actually the results of immunisation in Nos. 7 to 14 were themselves controls for Nos. 1 to 6. In the same way Nos. 15 and 16 were controls for Nos. 7 to 14, but for

TABLE I

CROSS IMMUNITY TESTS BETWEEN STRAINS OF C.P.D. VIRUS

Immunising strains				*Test strains*					
Sheep No.	*Immunising strain of virus*	*Date immunised*	*Result*	*Date*	*Cyprus 1*	*Cyprus 2*	*Cyprus 3*	*English U.F.*	*English 2*
1	Cyprus 1	3.2.46	++++	18.3.46	±	±	+	±	
2	,, 1	10.2.46	++++	18.3.46	±	O	O	±	
3	,, 2	10.2.46	++++	18.3.46	+	O	±	+	
4	,, 2	10.2.46	++++	18.3.46	O	O	O	+	
5*	,, 2	10.2.46	++						
6	,, 3	10.2.46	++++	18.3.46	+	+	O	+	
7	,, 1	1.4.46	++++	21.5.46	O	O	O	O	
8	,, 1	1.4.46	++++	21.5.46	+	+	+	±	±
9	,, 2	1.4.46	++++	21.5.46	±	±	+	+	±
10	,, 2	1.4.46	++++	21.5.46	+	O	±	+	+
11	,, 2	1.4.46	++++	21.5.46	+	+	±	+	+
12	,, 3	1.4.46	++++ (severe reaction)	21.5.46	+	+	±	+	+
13	English U.F.	1.4.46	++++ (severe reaction)	21.5.46	+	+	±	±	±
14	English U.F.	1.4.46	++++ (severe reaction)	21.5.46	+	+	+	O	±
15	English 2	7.5.46	+++	15.6.46	±	±	±	±	±
16	English 2	13.5.46	+++	15.6.46	±	±	±	±	±
17	Control			21.5.46	++++	++++	++++	++++	++++
18	Control			15.6.46	++++	++++	++++	++++	++++

Interpretation of symbols :

++++ = confluent eruption.
+++ = semi-confluent eruption.
++ = few scattered pustules.
\+ = abortive pustules drying to scabs by seventh day.
± = few scattered abortive pustules drying to scabs on or before seventh day.
± = redness swelling but no abortive papules or pustules ; reaction fading rapidly to dry crusts before seventh day.
O = absence of reaction.
* febrile reaction and keratitis : killed.

the sake of certainty a fresh animal, No. 17, was also used. Similarly No. 18 served as control for Nos. 15 and 16.

It may be noted that No. 5 had a severe local reaction with high fever. On the 15th day after inoculation it developed coryza and acute keratitis in both eyes, leading to corneal ulceration. It refused food, wasted away and was killed when moribund on the 30th day. With the exception of the eyes, no lesions were found *post mortem* in the organs or tissues.

Sheep No. 3 gave a very poor take of only four pustules, but nevertheless produced complete immunity against the immunising strain (Cyprus 2) and partial immunity against the others.

In order to facilitate a more detailed analysis the following brief table has been constructed from Table I using symbols to express the results. It is, of course, realised that such a symbolic representation can be only approximately accurate, and it tends to give a rather too clear cut visual picture.

TABLE II

RESULTS OF THE CROSS IMMUNITY TESTS IN TABLE I

(EXPRESSED SYMBOLICALLY)

Immunising strains	*Test Strains*									
	Cyprus 1		*Cyprus* 2		*Cyprus* 3		*English U.F.*		*English* 2	
Cyprus 1	++	++	++	++	++	+	+	+		
	++	++	++	++	++	+	++	++	++	++
Cyprus 2	±	++	++	++	+	++	+	+		
	++	+	++	++	+	++	+	+	++	++
Cyprus 3		±		±		++		±		
	±	±	±	±	++	++	±	±	±	±
English U.F.	±	±	±	±	±	±	++	++	+	+
English No. 2	++	++	++	++	++	++	++	++	++	++

++ indicates complete immunity.

± to + indicate varying degrees from partial to strong immunity and correspond with " vaccinoid " takes in human revaccination for smallpox.

The results can be classified under three heads:—

Cyprus Strains Against Cyprus Strains. While Cyprus 1 (Sheep) and Cyprus 2 (Goat) are closely related, there is a suggestion that No. 1 is the more efficient immunising agent. Both immunise equally strongly against Cyprus 3 (sheep) which was provisionally labelled " Aphthous Stomatitis." On the other hand, while this strain is less efficient *vis-à-vis* the other two, there can be no doubt that it is strain of C.P.D. virus.

Cyprus Strains Against English Strains. There appears to be no appreciable difference between Cyprus 1 and 2, both of which produce strong to complete immunity. Cyprus 3 is again less effective.

English Strains Against English Strains. Both are closely related but English 2 appears to be more effective.

English Strains Against Cyprus Strains. While both are closely related to Cyprus 1 and 2 the evidence suggests that English 2 is perhaps the most effective of the five strains.

DISCUSSION

Considered as a whole these results are closely comparable to the earlier investigations of Glover (1928, 1932-33), but the immunity tests were more exacting as in all cases undiluted virus was used whereas Glover used varying dilutions ranging from 1 in 100 to 1 in 50,000. An examination of his protocols shows that in three of his experiments (1932-33) using strains "Sp." and "M.F." and the standard strains U.F., the cross immunity was not completely reciprocal, the immunity produced by strains "Sp.," "T.W." and "M.F." against the standard strain "U.F." being complete, whereas that produced by "U.F." *vis-à-vis* any of the other three was strong but not complete. Glover considered that these slight differences were due to the higher virulence of strains "Sp." and "T.W.," and that all were probably immunologically identical. This opinion is presumably based on the assumption that a positive correlation exists between virulence and immunising potency which may, of course, be correct in the case of C.P.D. virus. In Glover's series (1932-33) there is no evidence that the caprine strain from Cyprus (R.W.) was of high virulence, but while it produced complete immunity against the standard strain (U.F.), the latter gave only partial protection against R.W. In consequence Glover remarked that "the strain may be slightly different from the English sheep strains." In the present series Cyprus 1 and 2 were almost equally effective immunising agents, but while the former was responsible for a severe outbreak, the latter was isolated from "scabs from the lips of an otherwise healthy goat."

The hypothesis of a positive correlation between virulence and immunising potency is certainly not borne out as a general rule in virus immunity, as, for example, smallpox, yellow fever and rinderpest. Vaccinia virus protects equally well against either variola or alastrim viruses; the well-known avirulent strain of yellow fever virus (17 D), itself derived from a mild case of the disease, is effective against the most virulent strains of the virus, and the goat-adapted avirulent strains of rinderpest is highly effective against fully virulent strains.

An alternative hypothesis similar to Glover's explanation in the case of the Cyprus strain (R.W.), is adopted in the present paper. Outbreaks of the clinical disease or syndrome known as C.P.D. in either sheep or goats are caused by various strains of virus, some of which are immunologically identical, whilst others are related in varying degrees. Such strains irrespective of their antigenic constitution may vary considerably in virulence. There is no evidence

that such differences are related either to the animal host, in this case the sheep or goat, or to the geographical distribution of outbreaks of the disease.

There is insufficient evidence to determine whether these differences are of a quantitative or of a qualitative character. In favour of the first hypothesis is their close correspondence with the results obtained in cross immunity tests between variola, alastrim and vaccinia viruses (Horgan and Haseeb, 1939). In this paper it was concluded that the results could be best explained by postulating a common antigen, which varies quantitatively, present in maximum amount in vaccinia virus and in lesser amounts in variola or alastrim viruses. This theory has been criticised editorally (1940), but recent studies in the Stack Laboratories on the relationships of strains of alastrim virus from different sources (1946)* appear to be in its favour. It is, however, unsafe to press the analogy too far and the relationship between strains of C.P.D. virus might well be due to minor qualitative differences in the antigenic complexes. Either theory assumes that there is a major antigen or antigens in common. The only way in which more definite proof could be obtained would be by the laborious method of cross absorption tests with mixtures of virus and hyper-immune sera. A simpler method would be a series of cross protection tests using sera from convalescent animals, but Glover (1932-33) has found that such sera are usually low in antibody content. They would, therefore, be unlikely to detect minor antigenic differences in the various strains of virus.

Regarded from a practical standpoint, *viz.*, the preparation of a vaccine, the results indicate the desirability of selecting a "master strain" with the maximum antigenic potency.

SUMMARY

A series of cross protection tests in sheep between strains of contagious pustular dermatitis virus of ovine and caprine origin is described.

The results which are in accordance with the earlier work of Glover indicate that the cross immunity varies from strong to complete.

It is concluded that the clinical disease is caused by a series of strains of virus, some of which are immunologically identical while others are closely related.

It is suggested that the differences can be best explained on the basis of quantitative differences of an antigen or antigens, while at the same time minor qualitative antigenic differences cannot be excluded.

There is no evidence that these differences are related to the species of animal host or to the geographical distribution of the disease.

* Unpublished observations.

ACKNOWLEDGMENTS

We are much indebted to Dr. R. J. Rowe, Chief Veterinary Officer, Cyprus, and Mr. R. E. Glover for supplying infective material and strains of virus.

REFERENCES

Bennett, S. C. J., Horgan, E. S., and Haseeb, M. A. (1944). *J. comp. Path.*, **54**, 131.

———. (1940). *Brit. med. J.*, **11**, 228.

Glover, R. E. (1928). *J. comp. Path.*, **41**, 318 ; (1932-33). *Rep. Dir. Inst. anim. Path., Camb.*, **3**, 1.

Horgan, E. S., and Haseeb, M. A. (1939). *J. Hyg.*, **39**, 615.

[*Received for publication July 22nd,* 1946]

THE RELATIONSHIP BETWEEN THE VIRUS OF CONTAGIOUS PUSTULAR DERMATITIS AND A VIRUS ISOLATED FROM SHEEP PAPILLOMATOSIS BY SELBIE

By

E. S. Horgan and Mansour Ali Haseeb,

Stack Medical Research Laboratories, Khartoum

INTRODUCTION

Previous studies in Khartoum (Bennett, Horgan, and Haseeb, 1944) on the range of pathogenicity of the virus of contagious pustular dermatitis clearly showed that it could be readily transmitted to the local laboratory strain of rabbits. This conclusion was in marked contradistinction to the results obtained by the majority of workers which are briefly summed up by Glover (1931) that "apart from some isolated experiments by Lanfranchi and Altara, there is general agreement that the rabbit, an animal which is admirably suited to the detection of the virus of vaccinia, is entirely refractory to the virus of dermatitis." In fact this refractoriness has been generally accepted as the main criterion of differentiation between the allied viruses of dermatitis and goat pox.

Selbie (1944) has reported transmissibility and serial passages through rabbits of a strain of virus isolated by him in 1936 from papillomatous lesions on the lips and nostril of a lamb. This author considered that he was dealing with a strain of virus of contagious pustular dermatitis which in the later stages of the disease is capable of producing warty growths in some animals. On the rabbit the lesions evolved into marked papillomatous outgrowths which persisted for some weeks and in general were comparable to the natural papillomatous form of the disease in lambs. Later, owing to his failure to retransmit the virus from rabbits to sheep, Selbie (1945) did not appear quite satisfied with his previous identification and concluded: "It would therefore appear probable that the rabbit-passaged virus is closely related to the virus of contagious pustular dermatitis and perhaps differs only in its adaptability from other strains that have been isolated." The present authors were greatly interested in these papers, as the incubation periods, appearance of the lesions and their subsequent evolution were different from those obtained in Khartoum (*op. cit.*).

The failure of Glover (1944) to infect English rabbits with the strain of virus of contagious pustular dermatitis which readily infected Khartoum rabbits further obscured the issue. It was therefore considered desirable to repeat Selbie's experiments, and in particular to carry out cross immunity tests between his virus and the strain (obtained from Glover) previously used in Khartoum (*op. cit.*).

EXPERIMENTAL TECHNIQUE AND RESULTS

Breed of rabbits.—There can be little doubt that the breed of rabbits used for experimental infection with animal poxes and in particular

with contagious pustular dermatitis is of fundamental importance; otherwise it is impossible to explain the successful outcome of the previous transmission experiments in Khartoum with the strain of virus with which Glover failed to infect rabbits in England. It is a curious fact that while genetically "pure" strains of rodents like rats and mice are now a commonplace in the majority of bacteriological laboratories, little attention—judging at least from the absence of details in most papers—has so far been paid to the maintenance of similar "pure" strains of rabbits. Such a strain maintained and issued by some international authority comparable to the well-known "Swiss" strain of mice used for yellow fever work, would be of great help to all research institutes dealing with animal poxes.

The rabbits used in the Stack Laboratories are a pure albino strain selectively inbred for ten years and derived originally from albinos from a strain of "Belgian Hare" crossed with albino Angoras both of which stocks originally came from England.

Strains of virus

*Selbie virus**.—Three samples were received from Dr. Selbie with the following notes: —

(A) Lesions glycerolated 31.1.45, 19 days after inoculation, S.D. Rabbit 153 (B.S.I. Expt. 8/45) of the 11th rabbit passage. Previous rabbit passages: 1st from lamb 3, 21 days; glycerol 6 days; 2nd to 5th passage, 129 days; glycerol 642 days; 6th to 9th passage, 76 days; glycerol, 2,241 days; 10th passage 30 days.

(B) Lesions glycerolated 20.6.45, from S.D. Rabbit 159 (B.S.I. Expt. 62/45) of the 12th rabbit passage 20 days after inoculation with sample A after storage for 120 days.

(C) Lesions dried 6.11.44, 13 days after inoculation, from S.D. Rabbit 126 (B.S.I. Expt. 142/44) of the 23rd rabbit passage. Previous rabbit passages: up to rabbits 83, 84 of 17th passage, as already reported (Selbie, 1945), dried 267 days; 18th passage 17 days; glycerol, 194 days; 19th to 22nd passage, 61 days.

In rabbits inoculations were made by scarification on to the shaven flanks, two animals being used for each strain. The incubation periods in all rabbits were constant between 29 and 30 days. One of the pair inoculated with Sample (B) was killed on the third day after the appearance of the reaction, the inoculated areas of the skin removed, ground up and stored in 50 per cent. glycerol at 4° C. as stock material. A portion of the fresh material was used to inoculate two more rabbits and in these the incubation period was also 29 days. The appearance and evolution of the lesions on all animals were identical with those described by Selbie (1944).

Two sheep were inoculated with Selbie's materials (specimens A, B and C), each being applied to separate areas of the skin. Two more animals were inoculated with the fresh rabbit material as

* In the remainder of this paper the term Selbie's virus indicates the virus isolated by him, while the initials C.P.D. indicate the virus of contagious pustular dermatitis.

described above. All animals were closely observed for 60 days, but no sign of a take could be detected, thus confirming Selbie's results (*op. cit.*).

C.P.D. virus (English strain). Sample of dried virus received from Mr. Glover (November, 1945) from a strain U.F. isolated by him in England in 1927, and previously used for cross immunity tests in Khartoum in 1944 (*op. cit.*).

C.P.D. virus (Cyprus strains). Three strains recently isolated (1946) from infective material sent from Cyprus by Dr. R. J. Rowe, Chief Veterinary Officer, Cyprus. Further details of these strains, including their immunological relationships, are discussed elsewhere in this issue (Horgan and Haseeb, 1947). In the present series they were used only in determining the susceptibility of rabbits and not for cross immunity tests.

The various strains were tested on rabbits and sheep. The English strain was inoculated on nine rabbits and two sheep. The Cyprus strains, after a preliminary sheep passage, were inoculated on to two rabbits, each strain being passed separately.

The incubation periods of all strains on both rabbits and sheep were very constant at three to four days. The appearance and evolution of the lesions were identical with those previously described by Bennett, Horgan and Haseeb (*op. cit.*). An interesting feature was the severe reactions on some of the rabbits inoculated with the fresh Cyprus strains.

SPECIFICITY OF THE LESIONS (C.P.D.) ON RABBITS

In the previous work in Khartoum (*op. cit.*) no attempt was made to passage back the virus from rabbits to sheep as it was assumed that the lesions were specific.

However, in view of the marked difference in the appearances of these lesions compared to those produced by Selbie's virus, it might be contended that the Khartoum results represented merely some non-specific reaction produced by the trauma of inoculation. To prove their specificity, a few cross immunity tests were carried out on rabbits and sheep.

The dried scabs as received from England were passed to a sheep and gave rise to typical vesico-pustular lesions. Two rabbits inoculated from this sheep showed an erythematous-squamous reaction, material from which produced typical lesions in a normal sheep, but no sign of a take in a sheep previously immunised with C.P.D.

The results of further cross immunity tests are summarised in Table I.

TABLE I

CROSS IMMUNITY TESTS IN RABBITS BETWEEN SELBIE'S AND C.P.D. VIRUSES

No. of animals used	*Immunising virus*	*Result*	*Interval after immunisation*	*Test virus*	*Result*
4	C.P.D.	++	29 days	Selbie	++
2	(Control)	—	—	Selbie	++
5	Selbie	++	68 days	C.P.D.	++
4	(Control)	—	—	C.P.D.	++

++ Indicates typical confluent take.

DISCUSSION

The above results which clearly indicate the differences between Selbie's virus and C.P.D. can be considered under three main heads, *viz.*, infectivity to sheep and rabbits, incubation periods and evolution of lesions, and immunological tests.

Selbie's claims of the infectivity of his virus for rabbits and its non-infectivity for sheep were fully confirmed.

There were marked differences between the viruses in both the incubation periods and the subsequent evolution of the lesions. The figures for Selbie's virus show an almost constant incubation period of 29 to 30 days, which was longer than that reported by Selbie (1945) irrespective of whether the inocula had been stored or were fresh. Apart from the longer incubation period, these results also differ somewhat from those of Selbie, who found that with stored glycerolated material the incubation periods were longer—from eight to 19 days with an average of 14 days—as compared with periods of six to nine days with an average of 7·2 days for relatively fresh material (stored for eight to 14 days). It is possible that these differences were related to the different strains of rabbits used in England and Khartoum. On the other hand the incubation period of C.P.D. virus in either rabbits or sheep was constant at three to four days, irrespective of whether the strain had been long passaged (English, U.F.) or freshly isolated (Cyprus strains).

The results of the cross immunity tests, as set out in Table I, are clear cut and show that there is no evidence of any immunological relationships between the viruses.

It would appear from viricidal tests carried out by Selbie (1945) that some sera from lambs recovered from C.P.D. neutralised his virus when tested in rabbits, but he also records that in two other experiments he failed to demonstrate any viricidal properties. These results are inconclusive but might be provisionally accepted as evidence that the two viruses possess some minor antigen in common. Considered as a whole the present experiments leave no doubts in the authors' minds that Selbie's virus must be regarded as *sui generis* and unrelated to C.P.D.

While it is not within the scope of this paper to discuss in detail the source of Selbie's virus a few comments are perhaps apposite. As quoted above, Selbie favours the view that it is a strain of C.P.D. virus with a somewhat different adaptability to that of other strains. There is, however, the possibility that it was a virus of sheep origin which might have caused papillomatous lesions on the original lamb clinically indistinguishable from C.P.D. On either assumption it is difficult to visualise such a complete mutation that after one passage the virus ceased to be infective for its own host while remaining infective for an unrelated species of animal and in addition undergoing a radical immunologicial change. As far as the authors are aware no instance of such a sudden mutation of a virus has been previously reported. The alternative hypothesis which

would account for the ease of adaptability and serial passages in rabbits is that the strain of rabbits used carried some latent virus activated during the inoculations of C.P.D. virus.* On the whole the authors favour this hypothesis which appears to them to explain better the recorded results, but until some positive evidence of the existence of such a rabbit virus is forthcoming, it must also remain within the realms of speculation.

SUMMARY

A comparative study has been made of a virus isolated by Selbie from lamb papillomatosis with a strain of contagious pustular dermatitis.

Selbie's findings of the non-infectivity of his virus for sheep and its infectivity for rabbits have been confirmed.

Cross immunity tests between these viruses failed to show any common immunological relationships.

From the results of the infectivity tests, incubation periods, evolution of the lesions produced and cross immunity tests it is concluded that Selbie's virus is *sui generis* and unrelated immunologically to the virus of contagious pustular dermatitis.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. F. R. Selbie, Bland-Sutton Institute of Pathology, Middlesex Hospital, for supplying the specimens of his virus, and to Mr. R. E. Glover, of the National Institute for Medical Research, for supplying his standard strain of the virus of C.P.D. as well as to the helpful correspondence and interest that both of these workers took in our experiments.

REFERENCES

Bennett, S. C. J., Horgan, E. S., and Haseeb, M. A. (1944). *J. comp. Path.*, **54**, 131.

Glover, R. E. (1931). *Proc. 11th Internat. Vet. Congr.*, **3**, 29 ; (1944). *Personal Communication quoted by* Selbie, F. R. (1945). *op. cit.*.

Horgan, E. S., and Haseeb, M. A. (1947). *J. comp. Path.*, **57**, 1.

Selbie, F. R. (1944). *J. comp. Path.*, **54**, 161 ; (1945). *Brit. J. exp. Path*, **26**, 89.

* The suggestion was first made in a private letter to one of the authors (E. S. H.) by Mr. Glover.

[*Received for publication July 22nd*, 1946]

THE PROPHYLACTIC USE OF PHENANTHRIDINIUM 1553 AGAINST INFECTION WITH *T. CONGOLENSE* AND *T. VIVAX*

By

H. A. Crawshaw,

Colonial Veterinary Service

INTRODUCTION

A series of observations was carried out in the Bechuanaland Protectorate in order to ascertain whether or not phenanthridinium 1553 had any protective action against infection with strains of trypanosomes occurring in that territory, and, if so, how long this protective action would last. The cattle used in the work were selected from an area where there was no possibility of previous infection with trypanosomiasis. They were kept under observation for a considerable time and blood smears were examined daily for one month before the commencement of the experiments. With the exception of experiments 4 and 5, in which, owing to shortage of staff it was only possible to take smears once a week, thick blood smears were taken daily, except Sundays, and examined for trypanosomes. Although in many cases a dose of 1 mg./kilo has been found sufficient to effect a cure in animals suffering from trypanosomiasis, results were not invariably successful, and so a prophylactic dose of 2 mg./kilo was decided upon.

EXPERIMENTAL INVESTIGATION

Experiment No. 1

Five head of cattle, Nos, 22, 23, 24, 25 and 28 were selected, and Nos. 22, 23 and 24 were each injected on 14.9.45 with phenanthridinium 1553 at the rate of 2 mg./kilo estimated live weight. Nos. 25 and 28 were left uninoculated to act as controls. The following day the five head were sent into the fly belt where heavy catches of *G. mortisans* could always be made. The cattle were grazed and watered in this area for a period of nine days and then returned to the laboratory where blood smears were taken daily except Sundays for a period of five months. The first control (No. 25) became positive 13 days after first entering the fly belt, while No. 28 showed parasites after 19 days. In both cases *T. vivax* made its appearance first, followed some time later by *T. congolense* and thereafter both parasites could be found in smears with ease. The three treated animals remained in excellent condition and showed no parasites in the blood up to the time the observation was discontinued after a period of approximately five months. The two controls developed severe symptoms of trypanosomiasis and were treated with phenanthridinium 1553 on 7.11.45, that is, some six weeks after infection, at the rate of 1.5 mg./kilo body weight. Microscopical examination showed the blood to be clear of parasites after 24 hours, and none

was seen for the rest of the time of the experiment. Recovery of condition was rapid. (Table I.)

Table 1

Single Subcutaneous Injection of Phenanthridinium 1553

Experiment	*No.*	*Dose and route of administration*	*No. of days in fly belt*	*Blood smears*	*Remarks*
I	22, 23, 24	2 mg./kilo s/c.	9	Negative 206 days	Clinically normal
	25, 28	Controls	9	Positive *T. cong.* and *T. vivax*	Treated 1·5 mg./kilo. Cured
II	34, 36, 37	2 mg./kilo s/c.	14	Negative 167 days	Clinically normal
	41, 42	Controls	14	Positive *T. cong.* and *T. vivax*	2 injections 2 mg./kilo, 7 days' interval. Cured

Experiment No. 2

In order to be satisfied as to the accuracy of the previous observation and to eliminate all possibility that through a coincidence the three injected cattle were not struck by infected flies, the observation was repeated, using cattle Nos. 34, 36, 37, 41 and 42. The first three were injected on 2.11.45 with phenanthridinium 1553 at the rate of 2 mg./kilo, and the last two were left as controls. All five were driven into the fly belt on 3.11.45, where they remained for 14 days, and were then returned to the laboratory. Both controls showed *T. vivax* in blood smears on 19.11.45, while *T. congolense* made its appearance shortly afterwards. In no instance was a parasite found in a blood smear from any of the injected animals which remained in excellent condition, in marked contrast to the controls which became very emaciated and weak. These two animals were treated and recovery ensued. Each received two injections at the rate of 2 mg./kilo at an interval of seven days. (Table I.)

Experiment No. 3

As noted by all workers with phenanthridinium 1553, a swelling develops at the site of a subcutaneous injection which, according to Barnett (1946), may be fairly severe in "grade" cattle. In the writer's experience if the drug is injected subcutaneously in horses an unsightly skin slough occurs some weeks later which renders this route of administration impracticable in such animals. On account of these undesirable sequelae it was decided to carry the observations a step further in order to ascertain whether any significant difference could be detected in the prophylactic action of the drug when injected subcutaneously or intravenously in equal

doses. Heifer No. 43 was kept as a control, while Nos. 44 and 45 were each injected intravenously on 24.11.45 with a dose equivalent to 2 mg./kilo body weight and Nos. 46 and 47 each received a similar dose subcutaneously. Ten days later all five were inoculated intravenously with 5 c.c. of infected blood from No. 42 (experiment No. 2 above). On 10.12.45, after a period of only six days, the control (No. 43) gave a positive blood smear showing *T. congolense* and *T. vivax,* and continued to do so until treated on 5.1.46. The other four animals remained negative and in good condition until the observations were discontinued after 145 days in the cases of Nos. 45, 46 and 47, and 97 days in the case of No. 44, which died at that time from an undiagnosed condition. After having been positive for 26 days the control was treated on 5.1.46 by the intravenous administration of phenanthridinium 1553 at the rate of 2 mg./kilo and thereafter with two further injections at seven day intervals. This animal remained negative until 18.4.46 (102 days) when the observations were discontinued. From this experiment it is evident that for a period of ten days after the injection of phenanthridinium 1553 the protection afforded by the drug against infection with *T. congolense* and *T. vivax* is not dependent upon its route of administration. Either may be employed, but to overcome the undesirable sequelae consequent upon a subcutaneous injection the intravenous medication is the method of choice. Although thrombosis of the vein after an intravenous injection of phenanthridinium 1553, as reported by Barnett (1946), has been noted in some cases, in the writer's opinion the advantages of the intravenous route as a means of administering the drug outweighs this disadvantage. The experiment put the prophylactic action of phenanthridinium 1553 to a severe test in that the four protected animals overcame a large infective case of virulent blood ten days after the drug had been given, whereas the control showed trypanosomes in its blood after a period of six days. (Table II.)

TABLE II

COMPARISON OF SUBCUTANEOUS AND INTRAVENOUS ROUTES

No.	*Dose and route of administration*	*Interval between prophylaxis and injecting infective blood*	*Remarks*
44, 45	2 mg./kilo i/v.	10 days	Clinically normal
46, 47	2 „ s/c.	10 „	„ „
43	Control	5 c.c. blood only	Positive *T. congolense* and *T. vivax* within 6 days. Treated three injections 2 mg./kilo at weekly intervals. Cured

Experiment No. 4

In view of the promising results shown in experiment No. 1 a parallel series of observations was commenced at the time using ten

head of cattle in order to ascertain (*a*) whether cattle could be maintained free from infection in a fly belt if injected at intervals with the drug, and (*b*) the optimum interval at which injections should be given. Nos. 61 to 68 were given an initial dose of phenanthridinium 1553 on 24.10.45, while Nos. 69 and 70 were kept as controls. All the cattle were sent into the fly belt where they remained and were injected at the rate of 2 mg./kilo at intervals thereafter, as indicated in Table III.

In order to be certain that the cattle were actually in fly country with a sufficiently high density to ensure that the results of the experiment should not be unduly optimistic owing to the cattle not being repeatedly fly struck, a fly patrol was established along the route that the animals followed when grazing and going to water each day. During the 31 days that the patrol was in operation 281 flies were caught, giving a daily average of just over nine. Although the catches were not heavy they would indicate a sufficient density to ensure that the cattle were repeatedly struck.

Table III

Effect of Injecting Phenanthridinium 1553 at Intervals

No.	*Interval injected subsequent to initial dose*	*No. of injections*	*Period in fly belt with negative blood*	*Period after last injection before blood became positive*	*Type*
61	7 days	6	46 days (died)	Negative	—
62	7 „	6	41 „ „	„	—
63	14 „	4	70 „	28 days	*T. brucei*
64	14 „	4	105 „	63 „	„
65	21 „	3	70 „	28 „	„
66	21 „	3	70 „	28 „	*T. congolense*
67	28 „	3	134 „	77 „	U.D.
68	28 „	3	134 „	77 „	„
69	Control	—	49 „	—	*T. congolense* & *T. vivax*
70	„	—	42 „	—	*T. congolense* & *T. vivax*

Note.—U.D. = undiagnosed. These two cattle became positive after the writer had left the Bechuanaland Protectorate.

Both Nos. 61 and 62 were reported to be showing symptoms of illness two days after the fourth injection, *i.e.*, after 28 days. They appeared listless, stood about under the trees and refused to graze. Two further injections were given at weekly intervals. No. 62 died on December 3rd and No. 61 on December 8th. On no occasion were parasites found in the blood stream. No. 63 was reported sick on December 7th, and No. 64 on December 8th with similar symptoms to those shown by the previous cases, *viz.*, inappetence and dullness, while, in addition, No. 63 appeared to have some mental aberration as it moved around the kraal in an aimless manner, its head stretched out with its mouth open. On December 13th No. 65 after its third injection was seen to be grazing poorly,

but inappetence was not complete, while two weeks later No. 66 although it had shown no previous symptoms, was noticed to be suffering from slight photosensitisation over areas of unpigmented skin. At this point it was decided to suspend the injection of the cattle with the exception of Nos. 67 and 68, which received their third dose on December 20th. These two animals were in excellent condition and health and showed no symptoms of toxicity. After receiving their last injection, trypanosomes appeared in the blood-stream of Nos. 63, 65, and 66 within 28 days, in No. 64 within 63 days and in Nos. 67 and 68 within 77 days. In no case did an animal become positive during the period that it was receiving injections. In three instances (Nos. 63, 64, and 65) the infecting organism was *T. brucei,* unaccompanied by either *T. congolense* or *T. vivax*. Only in the case of No. 66 did *T. congolense* appear as early as the 28th day after the last injection. It would seem that the prophylactic action of phenanthridinium 1553 against *T. brucei* is less than against the other two types. This is to be expected in view of the findings of other workers that the curative action of the drug against *T. brucei* is limited. Subsequent to the three injections at monthly intervals Nos. 67 and 68 remained with the other cattle in the fly belt without further treatment, and both were found to be positive 134 days after being brought in and 77 days after receiving their last injection. The controls (Nos. 69 and 70) showed *T. congolense* and *T. vivax* in blood smears 49 and 42 days respectively after entering the fly belt and together with Nos. 63 to 68 were treated by injecting 1.0 g. of tartar emetic intravenously at weekly intervals until an apparent recovery was made.

DISCUSSION

The results obtained in the small series of experiments using phenanthridinium 1553 prophylactically would indicate that the drug has a marked protective action against both natural and artificial infection with strains of *T. congolense* and *T. vivax* as they occur in the Bechuanaland Protectorate. One injection at the rate of 2 mg./kilo live weight will protect a bovine against natural infection for at least 14 days, while three injections given at intervals of one month protected two head of cattle for a period of 134 days, that is, 77 days after the last injection at which time blood smears became positive. The shortest period that breakdowns occurred after cessation of injections was 28 days. The toxic action of phenanthridinium 1553 is apparently cumulative in its effect as the two head of cattle that received weekly doses died after six injections, while two that received three injections at fortnightly intervals became seriously ill but recovered when the injections were discontinued. One of the animals that received three injections at intervals of three weeks showed slight symptoms, while the second one of this group became photosensitive, but both recovered when the injections were stopped. No. 43, which received three injections at

weekly intervals, however, showed no marked toxic symptoms. It is interesting that this animal was young and weighed half as much as those in which toxic symptoms were noted. It received, of course, a proportionate dose. Bell (1945) found that 6 mg./kilo may be fatal when injected intravenously in one dose, 5 mg./kilo produces marked toxic symptoms, while 4 mg./kilo produces mild toxic effects. In the foregoing observations the two animals that died received 12 mg./kilo over a period of five weeks. Two which received 8 mg./kilo over a period of seven weeks showed definite toxic symptoms, while two which received 6 mg./kilo also over a period of seven weeks were slightly affected. The drug is evidently retained in the body for a considerable time, and it is suggested that it owes its prophylactic action to this fact. The appearance in the experimental cattle of *T. brucei* (an organism to which bovines are normally fairly resistant) is of interest. The fact that it is able to become established in cattle after the cessation of the injections is significant and would merit further investigation to determine whether this parasite would become exalted in virulence if both *T. congolense* and *T. vivax* are suppressed by the action of phenanthridinium. Work carried out in laboratory animals by the writer has shown that the closely allied trypanosome *T. rhodesiense* has a considerable resistance to the action of phenanthridinium 1553.

Further experiments are obviously desirable to determine how the prophylactic action of phenanthridinium 1553 may be best exploited. It is hoped that other workers will undertake large scale experiments to ascertain the period during which a small dose of the drug (1·0 mg./kilo) repeated every 14 days or at some other suitable interval, would protect cattle whilst grazing in fly belt. It is possible that this smaller dose would be insufficient to give rise to toxic symptoms, but would have the power to protect cattle against infection.

The value of the drug used prophylactically in a dose of 2·0 mg./kilo is evident and should the observations reported in this paper be confirmed in large scale experiments, the movement of cattle through fly belts would be practicable and markets at present inaccessible might well be brought within their reach. The maintenance of cattle within the vicinity of fly belts, by periodic injections of the drug in sub-toxic doses might be a practicable possibility should it be confirmed that *T. brucei* as well as *T. congolense* and *T. vivax* can be suppressed when cattle are actually being subjected to a course of injections, as the above observations would indicate.

SUMMARY

Phenanthridinium 1553 has a marked prophylactic action against *T. congolense* and *T. vivax* in cattle; its action against *T. brucei* appears to be less.

A single dose of 2 mg./kilo protected cattle grazing in heavy fly country for a period of at least two weeks. Two cattle grazing in

the fly belt and treated with a dose of 2 mg./kilo on three occasions at monthly intervals only showed trypanosomes in blood smears after 134 days.

The toxic effect of phenanthridinium 1553 appears to be cumulative. The prophylactic action of the drug is the same whether administered subcutaneously or intravenously.

ACKNOWLEDGMENTS

Thanks are due to the Director of the Teddington Research Laboratory for supplies of phenanthridinium 1553; to the Principal Veterinary Officer, Bechuanaland Protectorate, for facilities for conducting the investigation and for permission to publish the paper; to Dr. J. Carmichael for his advice during its preparation, and to Dr. U. von Backstrom for his assistance in connection with the experiments.

REFERENCES

Barnett, S. F. (1946). *Vet. Rec.*, **58**, 325.
Bell, F. R. (1945). *Ibid*, **57**, 449.

[*Received for publication July 4th*, 1946.]

STUDIES ON *CORYNEBACTERIUM RENALE*

III: PENICILLIN TREATMENT OF EXPERIMENTALLY INFECTED MICE

By

R. Lovell and E. Cotchin,

Research Institute in Animal Pathology, Royal Veterinary College

INTRODUCTION

It has been recorded in a previous paper (Lovell and Cotchin, 1946) that a pyelonephritis may be produced in mice by the intravenous inoculation of *Corynebacterium renale*, and that multiplication of the organism occurs in the kidney tissue. A proportion of the infected mice die, and *C. renale* may be isolated from the kidneys and urine; some of the survivors when killed at a later stage also show lesions of pyelonephritis. These observations make it possible to study the value of therapeutic agents in *C. renale* infection under controlled conditions. So far, trials of this nature have been limited to cattle suffering from the natural disease, but as such cases are usually chronic and may show periodical improvement clinically without treatment, the assessment of the value of a given form of treatment has to be made with reserve. It was to be expected that trials with penicillin would be undertaken because this substance is excreted in the urine after parenteral administration and because some corynebacteria are known to be sensitive to penicillin *in vitro*. Morcos (1945) recorded the apparent cure of one case of pyelonephritis in a cow after penicillin therapy, and Beck, De Mott and Boucher (1945) published their experiences with six cases. One of their strains of *C. renale* was sensitive to penicillin *in vitro* in a concentration of 0·025 units per c.c. All six cases showed clinical improvement with the disappearance of haematuria and other symptoms after intramuscular injections of 10 million units of the sodium salt of penicillin. Haematuria returned in one case and *C. renale* was found subsequently in the urine of two others. The remaining three appeared to have made complete recoveries, but the authors realised that at the time of publication it might be a little premature to assume actual cures.

It is unwise to apply too strictly the conclusions derived from experiments in mice to the natural disease in cattle, but the results of such trials should help in forming an opinion as to the use and limitations of penicillin therapy in pyelonephritis. A brief report is therefore given of the *in vitro* sensitivity to penicillin of strains of *C. renale* and of the effect of penicillin therapy in mice infected with this organism.

SENSITIVITY OF *C. renale* TO PENICILLIN *in vitro*

Dilutions of a saline solution of the sodium salt of penicillin were prepared in tubes of broth, each of which contained 2·25 c.c. The concentration of penicillin in each c.c. of media ranged from 100 to 0·000,01 units. All the tubes, together with one control for each strain of organism tested, were inoculated with one loop full of a 24 hours' broth culture of the test strain. Opacity readings were made after 24 hours' incubation at 37° C. In all cases the control tubes showed adequate growth. The results obtained with 12 strains are given in the form of a frequency table.

TABLE I

SENSITIVITY OF STRAINS OF *C. renale* TO PENICILLIN *in vitro*

Degree of sensitivity (units per c.c.)	0·1	0·01	0·001	0·0001	
Number of strains	1	1	6	4	Total 12

A strain of staphylococcus was included in the test series and was sensitive to 0·01 units of penicillin per c.c., while a strain of *Bact. coli* failed to grow adequately in the presence of 100 units, but grew readily in the tube containing 10 units of penicillin per c.c. Although *C. renale* is to be regarded as sensitive to penicillin *in vitro* it is clear that the sensitivity may vary from strain to strain, ranging from 0·1 to 0·0001 units per c.c. with the 12 strains examined.

ACTIVITY OF PENICILLIN IN MICE EXPERIMENTALLY INFECTED WITH *C. renale*

Three experiments were made. In the first, 80 mice were inoculated intravenously with a saline suspension of a strain of *C. renale* sensitive *in vitro* to 0·001 units of penicillin per c.c.; the suspension was prepared by washing off cultures on agar slopes grown for 18 hours at 37° C. Each dose was computed to contain about 12 million living bacteria. The mice were divided into four groups of 20: Group A received no further treatment; Group B received 140 units of the sodium salt of penicillin subcutaneously twice daily on the first, second and third days following inoculation; Group C received similar treatment on the third, fourth and fifth days; Group D were treated in a similar manner on the fifth, sixth and seventh days. A cultural examination was made of one kidney and the urine, and a histological examination of the other kidney, of all mice which died. The survivors were killed on the 10th day and similar examinations made. The results of the cultural examinations obtained in this experiment are given in Table II.

TABLE II

RESULTS OF CULTURAL EXAMINATION OF MICE WHICH DIED AND OF THE SURVIVORS KILLED ON THE 10TH DAY

Day of death	*Number of mice examined*							
	Group A		*Group B*		*Group C*		*Group D*	
	+	—	+	—	+	—	+	—
0								
1	2		1		1		3	
2	6		6		4		5	
3	1		1				2	
4	1	1			1	1		
5							1	
6	1						1	
7								
8								
9	2					1		
10						1		
10 (S)	1	5		12	4	7	2	6
Totals	14	6	8	12	10	10	14	6

\+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.
Group A = Controls.
Group B = Penicillin 1st, 2nd and 3rd days.
Group C = Penicillin 3rd, 4th and 5th days.
Group D = Penicillin 5th, 6th and 7th days.
S = Survivors killed and examined on the 10th day.

Since some of the mice in the treated groups died before the administration of penicillin was completed, comparisons are made between the infection rates of those mice in the control group and those in the treated groups which were alive on the day the penicillin treatment was ended. If the infection rates of the dead and survivors of Group A and B from the third day, Groups A and C from the fifth day and Groups A and D from the seventh days are compared the figures given in Table III are obtained. A χ^2 test has been applied in each case and is also recorded.

TABLE III

COMPARISON OF THE CULTURAL EXAMINATION OF THE GROUPS OF MICE ALIVE ON PARTICULAR DAYS AND RESULTS OF χ^2 TEST

Day	*Group*	*Number of mice examined*			χ^2
		+	—	*Total*	
3rd	A	5	6	11	6·9
	B	0	12	12	
5th	A	4	5	9	0·43
	C	4	9	13	
7th	A	3	5	8	0·29
	D	2	6	8	

\+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.

This χ^2 test shows that in one series only is there statistical evidence that the administration of penicillin influenced the infection rate. The comparison between Group A (the control group) and Group B (the group which received penicillin twice daily on the first, second and third days) shows that the results obtained vary more than might be expected to occur by chance as a χ^2 of 6.9 with n = 1 gives a probability of less than 0·01. There is no evidence that penicillin given twice daily on the third, fourth and fifth days or on the fifth, sixth and seventh days influenced the observed results.

The second experiment varied slightly in certain details. Eighty mice were injected intravenously with a living suspension of *C. renale* prepared by the centrifugation of a 12 hours' broth culture. The strain was sensitive *in vitro* to penicillin in a concentration of 0·1 units per c.c. As before, the mice were divided into four groups of 20: Group A were controls and received no further treatment; Group B received 1,000 units of penicillin intraperitoneally immediately after the injection of *C. renale;* Group C received 1,000 units the following day, and Group D received two doses of 1,000 units, the first immediately following *C. renale* administration and the second the following day. The survivors were kept till the 21st day when they were killed. All mice were examined culturally and histologically, as in the previous experiment, and the results of the cultural examinations are given in Table IV.

TABLE IV

RESULTS OF CULTURAL EXAMINATION OF MICE WHICH DIED AND OF THE SURVIVORS KILLED ON THE 21ST DAY

Day of death	*Number of mice examined*							
	Group A		*Group B*		*Group C*		*Group D*	
	+	—	+	—	+	—	+	—
0								
1	5		1		1			
2								1
6			1					
7	1		1					
8	1				1			1
9	1			1		1		
13	3				1		1	1
14					1			
16			1					
20	1							
21					1			
21 (S)	2	6	1	14	7	7	2	14
Totals	14	6	5	15	12	8	3	17

+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.
S = Survivors, killed and examined on the 21st day.
Group A = Controls.
Group B = Penicillin on O day.
Group C = Penicillin on 1st day.
Group D = Penicillin on O and 1st days.

If similar comparisons and calculations are made as with the first experiment then the infection rates of the dead and survivors of Groups A and B from 0 day, Groups A and C from the 1st day, and Groups A and D from the 1st day, are those given in Table V.

TABLE V

COMPARISON OF THE CULTURAL EXAMINATION OF THE GROUPS OF MICE ALIVE ON PARTICULAR DAYS AND RESULTS OF χ^2 TEST

		Number of mice examined			
Day	*Group*	+	—	*Total*	χ^2
0	A	14	6	20	8·1
	B	5	15	20	
1	A	9	6	15	0·015
	C	11	8	19	
1	A	9	6	15	7·7
	D	3	17	20	

+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.

Two of the series in this experiment show by the χ^2 test that the administration of penicillin influenced the results obtained. The group receiving one dose of penicillin at the time of injection, and the group receiving penicillin at the same time and also on the following day when compared with the control group give χ^2 figures of 8·1 and 7·7 respectively. In both cases P is less than 0·01 and the results may therefore be regarded as significant. There is no evidence that the mice receiving the one dose of penicillin one day after the injection of *C. renale* enjoyed any advantage.

Sixty mice were used in the third experiment, and they received an intravenous inoculation of a 12 hours' broth culture of *C. renale* concentrated by centrifugation. This strain was sensitive *in vitro* to penicillin in a concentration of 0·001 units per c.c. The mice were divided into three groups of 20: Group A were control mice and received no further treatment; Group B received 500 units of penicillin subcutaneously immediately after the inoculation of *C. renale* and thereafter 500 units daily for seven days; Group C were given penicillin in the drinking water from the day of injection to the seventh day, the dose being computed at 500 units daily per mouse. The mice which died and the survivors which were killed on the 14th day were examined bacteriologically and histologically as in the former experiments. The results of the cultural examinations are given in Table VI.

As with the other experiments, the infection rates of Groups B and C were compared with that of Group A from the 7th day when penicillin therapy terminated. The relevant data with the results of the χ^2 tests given in Table VII show that penicillin was of value

TABLE VI

RESULTS OF CULTURAL EXAMINATION OF MICE WHICH DIED AND OF THE SURVIVORS KILLED ON THE 14TH DAY

Day of death	Number of mice examined					
	Group A		Group B		Group C	
	+	—	+	—	+	—
0						
1						
2	1					
6						
7	3					
8	1					
9	3					1
10	1					
11						
12	1				1	
13						
14	1				1	
14 S.	3	6	3	17	8	9
Totals	14	6	3	17	10	10

+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.
S = Survivors killed and examined on the 14th day.
Group A = Controls.
Group B = Penicillin subcutaneously from 0 to 7 days.
Group C = Penicillin in the drinking water from 0 to 7 days.

TABLE VII

COMPARISON OF THE CULTURAL EXAMINATION OF THE GROUPS OF MICE ALIVE ON THE 7TH DAY AND RESULTS OF χ^2 TEST

Day	Group	Number of mice examined			χ^2
		+	—	Total	
7th	A	10	6	16	8·7
	B	3	17	20	
7th	A	10	6	16	0·56
	C	10	10	20	

+ = *C. renale* recovered from urinary tract.
— = *C. renale* not recovered.

in Group B but not in Group C; a figure of 8·7 gives a probability of less than 0·01, whereas 0·56 gives a probability of more than 0·3 On the other hand, there is a suggestion displayed in Table VI that there may have been some delay in the deaths in Group C; but the infection rate is a better indication of the effect of any specific form of therapy in this type of disease.

It is possible that some of the kidneys which showed histological damage did not contain living *C. renale,* and we have so far

considered the recovery of *C. renale* by cultural methods only in assessing the value of penicillin therapy. Kidneys from the mice in all three experiments were, however, examined histologically, and in general the results were in accordance with the cultural examinations. In a few mice from which *C. renale* was not recovered there was evidence of damage to the kidney tissue, and if these are also counted as infected similar deductions are made in each experiment. The χ^2 figures for the three groups in the first trial become 7·7, 1·7 and 2·3 respectively as compared with 6·9, 0·43 and 0·29 already recorded; in the second trial the figures are 12·1, 0·14, and 8·1 as compared with 8·1, 0·015 and 7·7 respectively, whilst in the third trial the figures are 10·89 and 0·42 as compared with 8·7 and 0·56 respectively. It is, therefore, clear that if these figures are accepted the same conclusions can be drawn, namely, that in the first trial penicillin had a beneficial effect in Group B alone, in the second trial in Groups B and D and in the third trial in Group B alone.

DISCUSSION

As judged by the infection rates in mice there appears to be some advantage when mice are given penicillin subcutaneously twice daily during the first three days after infection or when a single dose is administered intraperitoneally at the time of inoculation. Beneficial effects are also observed when penicillin having been administered at the time of inoculation is repeated the following day or for the following seven days. On the other hand, no advantage is obtained when administration is delayed till the third or fifth day; neither is there any evidence that one large dose given the day after infection is of value.

Moderate dosage of penicillin given in the drinking water for seven days did not influence the infection rate, although this method of administration has been used with some success in experimental infection of mice with *Erysipelothrix rhusiopathiae* by Harvey *et al.* (1945), who gave doses of about 5,000 units per mouse daily. Oral administration was also successful in experimental streptococcal infection in mice; White *et al.* (1946) gave single doses of the order of 16,000 to 17,000 units, and thereby protected 29 of the 30 injected. These are large doses compared with those we have given.

The results we have obtained in our trials may have been influenced by the variation in the sensitivity of different strains to penicillin. Those used in the first and third tests were equally sensitive, whereas that used in the second was more resistant. It is unlikely that this factor affected the results to the same extent as did the number of injections. It is generally held that several doses of penicillin given over a short period are of more value than a single dose, even though the latter dose may be a large one. This is undoubtedly due to the fact that penicillin is excreted rapidly after

parenteral administration, and repeated doses are necessary to maintain a sufficient concentration in the tissues.

The results obtained show that penicillin is of some value in the treatment of experimental pyelonephritis in mice when given early in the disease, but it cannot be assumed that field trials in cattle will yield results similar to those obtained with mice. There are, moreover, no grounds for optimism as to a permanent cure being effected once the kidneys have suffered considerable damage. In mice there is histological evidence of damage to the kidney tissue as early as 30 hours after the intravenous inoculation of *C. renale* (Lovell and Cotchin, 1946), and there appears to be less likelihood of penicillin having a favourable effect once this has occurred. In cattle, the natural disease is not diagnosed until considerable changes have developed in the urinary tract and there is some obvious abnormality in the urine. Until it is possible to diagnose pyelonephritis in cattle in its early stages we feel that the chances of permanent cure may not be very great, and the observations made herewith support the cautious attitude adopted by Beck, De Mott and Boucher (1945). They conclude from their trials with six cases of the natural disease that "penicillin sodium is of some definite value in the treatment of infectious cystitis and pyelonephritis of cows, but due to the chronicity and tendency for cases to improve clinically without treatment it might be a little premature to assume actual cures."

SUMMARY

Strains of *C. renale* vary in their sensitivity *in vitro* to penicillin.

Penicillin is of value in the treatment of experimentally produced *C. renale* pyelonephritis in mice when given early in the disease.

REFERENCES

Beck, J. D., De Mott, T. and Boucher, W. B. (1945). *Vet. Ext. Quart. Univ. Pa.*, **100**, 10.

Harvey, P. C., Libby, R. L. and Waller, B. B. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 307.

Lovell, R. and Cotchin, E. (1946). *J. comp. Path.*, **56**, 205.

Morcos, Z. (1945). *Vet. Rec.*, **57**, 425.

White, H. J., Lee, M. E. and Alverson, C. (1946). *Proc. Soc. exp. Biol, N.Y.*, **62**, 35.

[*Received for publication, August 9th,* 1946.]

THE ASCORBIC ACID CONTENT OF THE BLOOD SERUM OF FARM ANIMALS

By

M. M. Sheahan,

Veterinary College of Ireland

INTRODUCTION

Domestic animals are believed to be capable of synthesising in their bodies sufficient ascorbic acid for the maintenance of normal health, but it has been suggested that a relative deficiency of this substance may exist in farm animals, which, although it produces no definite symptoms of disease, may have a deleterious effect on reproductive function.

A possible connection between ascorbic acid and reproductive function was indicated by the investigations of Biskind and Glick (1936), who found that the concentration of ascorbic acid in the corpus luteum was at its maximum when this organ was fully developed, and that there was an apparent correlation between the amount of ascorbic acid in the corpus luteum and the production of progesterone. Phillips, Lardy, Heizer and Rupel (1940) estimated the ascorbic acid content of the blood plasma and semen of a number of sterile bulls, and those which showed a low concentration in both plasma and semen were treated by subcutaneous injections of ascorbic acid. It was concluded that although such treatment is not always successful, it may be beneficial in some cases, particularly in bulls not fully mature. Phillips, Lardy, Boyer and Werner (1941) determined the amount of ascorbic acid in the blood plasma of cows in an attempt to correlate it with sterility and, in cases where a relative deficiency might exist, to increase the concentration by subcutaneous injection of this agent. Cows with regular oestrus cycles and showing no abnormalities of the reproductive tract, but which failed to conceive, seemed to be most amenable to this treatment.

The Cow. Gossee (1936) found the ascorbic acid content of the serum of 53 pregnant cows to range between 1·02 to 3·80 mg. per 100 c.c.*, and that the amounts were slightly higher in pregnant than in non-pregnant animals. Phillips, Lardy, Boyer and Werner (1941) found the average amount in the plasma of 154 cows from February to April to be 0·39 with a range of 0·19 to 0·66. They stated that the concentration was higher during oestrus than in dioestrus and that, generally speaking, there was no difference between good and poor breeders in their peak concentrations during oestrus. Radeff (1941) found the average content of the serum of 130 dairy cattle to be 1·32± 0·02 with a range of 0·79 to 2·64, and Bortree, Huffman and Duncan (1942), who examined the plasma of 24 cows, obtained an average of 0·44 with a range of 0·11 to 0·80. These workers estimated the ascorbic acid content of the plasma of a group of cows over a period of 8 to 10 days. They found that marked variations occurred from day to day, and even that differences occurred in samples taken from the same animal at three-hourly intervals.

* Throughout this article the figures quoted for ascorbic acid are given in mg. per 100 c.c.

The Bull. Phillips, Lardy, Heizer and Rupel (1940) examined the blood plasma of 11 sterile bulls and found the average ascorbic acid content to be 0·20 with a range of 0·08 to 0·32, while Ray (1941) in his study of the effects of disease on the ascorbic acid content of the blood serum of Indian Hill bulls obtained an average of 0·50 with a range of 0·34 to 0·77.

The Sheep. Radeff (1941) found the average content of the serum of 125 sheep to be 0·91± 0·02 with a range of 0·40 to 1·56, while Rasmussen, Cole, Miller and Thorp (1944) examined the plasma of 31 ewes and obtained an average of 0·40± 0·21 with a range of 0·08 to 1·03.

The Pig. Radeff (1941) found the average ascorbic acid content of the serum of 78 pigs to be 1·06 ± 0·03 with a range of 0·6 to 1·90.

The Ascorbic Acid Content of the Reproductive Organs. Biskind and Glick (1937) studied the ascorbic acid content of the testis of the rabbit and of the bull in relation to the anatomical and functional changes which occur in that gland. They stated that the interstitial tissue was relatively rich in ascorbic acid, and suggested that there might be a connection between this and the hormonal function of the gland.

Phillips (1940) found the ascorbic acid content of the semen of normal bulls to lie between 3·0 to 7·0, while the plasma concentration lay between 0·20 to 0·40. He associated amounts below this range with sterility due to a relative deficiency. In further work, Phillips and Lardy (1940) determined the ascorbic acid content of potent bull semen and found it to lie between 1·5 to 3·5. They concluded that amounts below 1 mg. were indicative of an ascorbic acid deficiency in the semen. Continuing this work, Phillips, Lardy, Boyer and Werner (1941) estimated the ascorbic acid content of the semen of 29 bulls; the average amount was 6·19 with a range of 3·0 to 8·0. In these cases amounts below 2 mg. were associated with sterility. Jurgens (1942) estimated the ascorbic acid in the semen of fertile and infertile bulls. The average content of the ejaculate was 0·26, of the testicular spermatozoa 0·52 and of the epididymal spermatozoa 0·92. He found no definite connection between the sperm count and the ascorbic acid content of the semen.

Since it appears that in America a relative deficiency of ascorbic acid may be responsible for some cases of sterility, it was suggested that a similar deficiency might also be the cause of sterility in bulls in Ireland. This work was, therefore, undertaken to establish the mean and variations to be found in the ascorbic acid concentration in the blood serum of the cow, the bull, the sheep and the pig and also to determine whether a relative deficiency of ascorbic acid could be correlated with sterility in the bull.

MATERIAL

Most of the blood used in these estimations was obtained from the City of Dublin Corporation Abattoir. The blood serum of 250 cows was used whose ages ranged from three to seven years. Among the reasons for their slaughter were sterility, disease and loss of milk yield. Their condition varied from good to extreme emaciation. Samples from 125 bulls were studied ranging from ten months to seven years. They were slaughtered because of sterility, disease

and faulty conformation. Estimations were made on the blood of 200 sheep from one to three years old, and 150 pigs about 10 months. Both sheep and pigs were in good condition.

In order to simplify the calculations of the average amounts and variations of the ascorbic acid content of the blood serum, the estimations are grouped together in the tables so that each group shows the ascorbic acid content of the blood serum from 25 animals.

METHOD

To keep the loss of ascorbic acid to a minimum, analyses were made as soon as possible after the animals had been slaughtered, which in no case exceeded one to two hours. The blood samples were centrifuged immediately on their arrival at the laboratory, and the required amount of serum drawn off. It may be mentioned that earlier workers used either oxalated plasma or serum for their estimations, but this appears to be of little significance since the results obtained are in close agreement. In the present case it was more convenient to use serum since the blood samples were also required for other analyses which precluded the addition of oxalate.

At first the secretion of the testis and epididymis were analysed separately, but the amount of fluid from the latter varied and was often too small to make an accurate estimation possible. It was decided, therefore, to combine the fluids obtained from the two structures. The testicle and epididymis were sliced and the fluids squeezed out; the same method was used for the prostate gland. The seminal vesicles were ligatured on removal from the carcase to prevent loss of their contents during transit to the laboratory.

The serum or fluid expressed from the reproductive organs was diluted in the proportion of 1 c.c. of serum or fluid, 1 c.c. of distilled water, and 1 c.c. 20 per cent. trichloracetic acid, and then filtered: 1·5 c.c. of this filtrate was titrated against a 1 : 500 solution of 2·6 dichlorophenolindophenol, the end-point being the first pale pink colour. A 1 : 500 solution of standardised 2·6 dichlorophenolindophenol is the equivalent of 0·002 mg. ascorbic acid.

RESULTS

Blood Serum. The figures set out in Table I show that the ascorbic acid content of the blood serum of cows fell from the end of October, when the average was 0·55, until the beginning of January, when it was 0·26. At the same time the minimum values decreased from 0·26 to 0·92 to 0·14 to 0·40. The range was great when the work was started in October, but later became more limited. The lowest concentrations were found at the beginning of January, after which there was a tendency for the average, minimum and maximum values to increase until April, when the average amount was 0·50 with a range of 0·40 to 0·64.

TABLE I
Cows (25 animals in each group)

Group	*Mean mg. per 100 c.c.*	*Range mg. per 100 c.c.*	*Standard deviation*	*Dates between which samples were taken*
1	0·55	0·26–0·92	0·15	Oct. 23rd to Nov. 2nd, 1945.
2	0·43	0·20–0·70	0·12	Nov. 2nd to Nov. 8th, 1945.
3	0·40	0·24–0·60	0·08	Nov. 8th to Nov. 12th, 1945.
4	0·26	0·14–0·40	0·06	Jan. 8th to Jan. 17th, 1946.
5	0·28	0·20–0·42	0·06	Jan. 17th to Jan. 28th, 1946.
6	0·32	0·22–0·44	0·05	Jan. 28th to Jan. 30th, 1946.
7	0·29	0·20–0·44	0·05	Jan. 30th to Feb. 1st, 1946.
8	0·39	0·30–0·52	0·06	Feb. 11th to Feb. 13th, 1946.
9	0·39	0·30–0·54	0·05	Mar. 7th to Mar. 13th, 1946.
10	0·50	0·40–0·64	0·06	Apr. 9th to Apr. 15th, 1946.
	0·38	0·14–0·92		

Table II shows a fall in the ascorbic acid content of the blood serum of bulls from 0·48 with a range of 0·20 to 0·98 in Group 1 examined in October/November to the lowest minimum and maximum values of 0·18 to 0·52 in Group 3 in December, but the lowest average amount was found in Group 4 between January and March. There was an increase in the average and minimum values in the next group of estimations made during March and April.

TABLE II
BULLS (25 animals in each group)

Group	*Mean mg. per 100 c.c.*	*Range mg. per 100 c.c.*	*Standard deviation*	*Dates between which samples were taken*
1	0·48	0·20–0·98	0·17	Oct. 22nd to Nov. 27th, 1945.
2	0·42	0·26–0·60	0·09	Nov. 27th to Dec. 6th, 1945.
3	0·36	0·18–0·52	0·08	Dec. 6th to Dec. 20th, 1945.
4	0·36	0·22–0·54	0·09	Jan. 17th to Mar. 6th, 1946.
5	0·40	0·26–0·52	0·06	Mar. 7th to Apr. 28th, 1946.
	0·40	0·18–0·98		

Table III shows that there was a slight fall in the average amount of ascorbic acid in the blood serum of sheep from December to January, when the lowest amounts for the mean, minimum and maximum values were found. During January estimations were made on two further groups of 25 sheep; in the first of these the figures were slightly higher than in the second group. After that there was a slight but steady rise until April.

TABLE III

SHEEP (25 animals in each group)

Group	*Mean mg. per 100 c.c*	*Range mg. per 100 c.c.*	*Standard deviation*	*Dates between which samples were taken*
1	0·34	0·20–0·42	0·06	Dec. 5th to Dec. 13th, 1945.
2	0·33	0·22–0·44	0·07	Dec. 17th to Dec. 19th, 1945.
3	0·26	0·14–0·40	0·06	Jan. 8th to Jan. 17th, 1946.
4	0·31	0·18–0·44	0·06	Jan. 17th to Jan. 28th, 1946.
5	0·30	0·16–0·40	0·05	Jan. 28th to Feb. 1st, 1946.
6	0·37	0·28–0·46	0·05	Feb. 11th to Feb. 13th, 1946.
7	0·39	0·22–0·54	0·07	Mar. 4th to Mar. 6th, 1946.
8	0·44	0·34–0·54	0·06	Apr. 15th to Apr. 17th, 1946.
	0·34	0·14–0·54		

Table IV shows that the first and lowest estimations of the ascorbic acid content of the blood serum of pigs were obtained at the beginning of January, after which there was a slight but steady rise until March. April shows a slight fall in the mean value although the range of 0·28 to 0·54 is the same as that of the previous month.

TABLE IV

PIGS (25 animals in each group)

Group	*Mean mg. per 100 c.c*	*Range mg. per 100 c.c*	*Standard deviation*	*Dates between which samples were taken*
1	0·24	0·16–0·32	0·04	Jan. 10th to Jan. 11th, 1945.
2	0·33	0·20–0·58	0·08	Jan. 11th to Jan. 21st, 1946.
3	0·34	0·26–0·48	0·05	Jan. 21st to Jan. 30th, 1946.
4	0·34	0·20–0·46	0·07	Jan. 30th to Feb. 1st, 1946.
5	0·38	0·28–0·54	0·07	Mar. 6th to Mar. 13th, 1946.
6	0·37	0·28–0·54	0·05	Apr. 4th to Apr. 15th, 1946.
	0·33	0·16–0·58		

Serum, Testis and Epididymis. Estimations of the ascorbic acid content of the blood serum and of the fluids from the testis and epididymis from 50 bulls were made. Unfortunately it was not possible to ascertain the reasons for the slaughter of these animals as they had been bought in the open market, but from the data collected *post-mortem* it is probable that in many cases sterility was the primary reason. In young bulls it is likely that failure to obtain the licence of the Department of Agriculture on the grounds of faulty conformation was the cause. The results of the estimations are given in Tables V and VI.

TABLE V

THE ASCORBIC ACID CONTENT OF THE BLOOD SERUM AND SECRETIONS OF THE TESTIS AND EPIDIDYMIS OF BULLS (February 25th to March 22nd)

Ref No.	*Date*	*Blood serum*	*Testis and Epididymis*	*Remarks*
620	February 25th, 1946	0·48	2·44	
621		0·54	1·63	
622		0·46	1·72	
623	February 28th, 1946	0·50	4·01	
624		0·40	2·27	Two-year-old. Adhesions. Tb lesion.
625		0·50	1·71	
648	March 6th, 1946	0·36	3·36	
669	March 7th, 1946	0·36	2·16	1½-year-old.
670		0·38	2·44	Two-year old. Adhesions.
684	March 8th, 1946	0·50	2·72	Two-year-old.
685	March 12th, 1946	0·36	1·55	10 months old. Fibrosis of one testicle.
686		0·46	1·15	1 year old.
687		0·42	1·60	1 year old.
688		0·40	1·28	1 year old.
689		0·52	1·31	1 year old.
711	March 20th, 1946	0·36	3·70	7 year old.
712	March 21st, 1946	0·38	1·88	Adhesions.
713		0·40	1·85	Adhesions. Fibrosis of both testicles.
714		0·34	1·41	6 year old.
715	March 22nd, 1946	0·36	1·92	
716		0·26	1·35	
717		0·42	1·37	
718		0·44	2·83	
719		0·40	1·47	
720		0·40	1·56	
		10·40	50·69	
Mean		0·42	2·03	
Standard deviation		0·06	0·76	
Range		0·26–0·54	1·15–4·01	

The concentration of ascorbic acid in the blood serum of these animals was within normal range when compared with figures quoted from various sources. The concentration in the combined fluids of testis and epididymis showed great variation and appeared to bear no relationship to the amount in the blood serum. It was evident from the high percentage of defective spermatozoa produced by some of these bulls and the destruction of spermatogenic tissue found on histological examination that the animals were sterile. The appearance of the testis and the frequent occurrence of fibrous adhesions between the parietal and visceral layers of the tunica vaginalis indicated long-standing inflammatory lesions.

Thus, in Bull No 648 (Table V), the content of the blood serum was 0·36 where the average amount was 0·42 (± 0·06) with a range of 0·26 to 0·54, and that of the fluids from the testis and epididymis was 3·36 where the average was 2·3 (± 0·76) with a range of 1·15

to 4·01. The histology of the testis of this bull showed degeneration and vacuolation of the spermatogenic tissue layer, while the number of defective spermatozoa found in smears prepared from the fluid of the epididymis was 47 per cent. Similarly in Bull No. 731

TABLE VI

THE ASCORBIC ACID CONTENT OF THE BLOOD SERUM AND FLUIDS OF THE TESTIS, AND EPIDIDYMIS, PROSTATE GLAND AND SEMINAL VESICLES OF BULLS
(March 27th to April 11th)

Ref No.	*Date*	*Blood serum*	*Testis and epididymis*	*Prostate gland*	*Seminal vesicles*	*Remarks*
721	March 27th, 1946	0·34	1·97	1·56	—	5 year old. Adhesions.
722		0·38	1·38	—	—	
723		0·30	1·37	1·04	—	1 year old. Thin watery secretion.
724	March 28th, 1946	0·38	2·40	1·08	—	
725		0·48	1·65	—	—	Adhesions.
726		0·50	0·93	0·78	—	Extensive adhesions.
727		0·40	1·08	—	—	
728		0·40	1·31	0·80	—	Tb. Epididymis.
729		0·40	1·11	0·88	—	
730		0·40	1·41	—	—	
731	April 3rd, 1946	0·40	2·02	0·60	1·60	
732		0·34	1·74	0·44	—	
733		0·38	1·70	0·80	—	
734	April 4th, 1946	0·40	1·25	0·91	1·37	Adhesions.
735		0·42	1·32	0·91	—	
736		0·36	1·69	0·81	1·00	
737		0·40	1·75	0·64	—	Adhesions. Tb. Epididymis.
787	April 11th, 1946	0·62	2·09	0·64	0·76	Adhesions.
788		0·50	1·63	0·80	0·92	Adhesions.
789		0·48	1·19	—	—	
790		0·58	1·63	0·50	0·92	Extensive adhesions.
791		0·50	1·34	1·08	0·96	Very extensive adhesions.
792		0·54	1·33	—	—	
793		0·56	1·01	0·60	0·88	
794		0·44	1·42	0·54	—	Adhesions.
		10·90	37·72	15·41	8·41	
Mean		0·44	1·51	0·81	1·05	
Standard deviation		0·08	0·35	—	—	
Range		0·30–0·62	0·93–2·40	0·44–1·56	0·76–1·60	

(Table VI) the serum content was 0·40, the group average being 0·44 (± 0·08) and the range 0·30 to 0·62, while the concentration in the fluids of the testis and epididymis was 2·02 where the average was 1·51 (± 0·35) with a range of 0·93 to 2·40. There was a relatively high concentration of 1·6 in the fluid of the seminal vesicles, while that of the prostate gland was low, being only 0·6. In this case also there was degeneration and vacuolation of the spermatogenic tissue layer and an increase in the amount of interstitial tissue. The

number of defective spermatozoa in the fluid obtained from the epididymis was 57 per cent.

In conclusion it is evident from the estimations recorded in this work that there is a tendency for the ascorbic acid concentration of the blood serum to fall during the winter months, but whether this is of any significance in connection with reproduction is not at all clear. Indeed, a severe functional disturbance of the reproductive organs of the bull may be present and yet the ascorbic acid content of both the blood serum and of the reproductive organs themselves remains within normal range.

SUMMARY

As a result of estimations carried out on 725 blood samples from different species, the average ascorbic acid content of the blood serum was found to be: (*a*) cow—0·38 with a range of 0·14 to 0·92 between October and April; (*b*) bull—0·40 with a range of 0·18 to 0·98 between October and March; (*c*) sheep—0·34 with a range of 0·14 to 0·54 between December and April; (*d*) pig—0·33 with a range of 0·16 to 0·58 between January and April.

There was a tendency for the amounts of ascorbic acid in the blood serum to fall during the winter months and to rise during the spring.

No correlation existed between the ascorbic acid content of the blood serum and that of the fluids of the reproductive organs.

There was evidence of marked reproductive dysfunction in the 50 bulls examined, but a relative deficiency in the ascorbic acid content of the blood serum or of the reproductive organs could not be established.

REFERENCES

Biskind, G. R., and Glick, D. (1936). *J. biol. Chem.*, **27,** 113; (1937). *Arch Path.*, **23,** 363.

Bortree, A. L., Huffman, C. F., and Duncan, C. W. (1942) *J. dairy Sci.*, **25,** 983.

Gossee, K. H. 1936). *Inaug. Diss*: Hanover.

Jurgens, F. (1942). *Inaug Diss*: Hanover. *Abst. Dtsch. tierärtzl. Wschr.*, **50,** 393.

Phillips, P. H. (1940). *J. Amer. vet. med. Ass.*, **97,** 165.

Phillips, P. H. and Lardy, H. A. (1940). *J. dairy Sci.*, **23,** 530.

Phillips, P. H., Lardy, H. A., Heizer, E. E., and Rupel, L. W. (1940). Ibid., 873.

Phillips, P. H., Lardy, H. A., Boyer, P. D., and Werner, G. M. (1941). *Ibid.*, **34,** 153.

Radeff, T. (1941). *Yeard. vet. med. Fac.* Sofia, *Abst. Jber. vet. Med.*, **68,** 196.

Rasmussen, R. A., Cole, C. L., Miller, M., and Thorp, F. (1944). *J. anim. Sci.*, **3,** 340.

Ray, S. N. (1941). *Ind. J. vet. Med.*, **11,** 78.

[*Received for publication, August 15th, 1946.*]

SEX HORMONES IN THE URINE OF PREGNANT CATTLE

By
L. B. O'Moore,
Veterinary College of Ireland.

INTRODUCTION

The work recorded in this paper is an investigation of the occurrence of sex hormones in the urine of the in-calf cow with special reference to their possible value as an aid to the diagnosis of pregnancy.

During pregnancy in woman chorionic gonadotrophin, pregnandiol and oestrogenic substances have been demonstrated in the urine. Of these, it has been established that chorionic gonadotrophin does not appear in detectable quantities in the urine of the cow at any time during pregnancy. Asdell and Madsen (1933) submitted both blood and urine of cows at all stages of pregnancy to the Ascheim-Zondek and the Friedman tests, and in no case was a positive result obtained, while Bruhn (1933) found that chorionic gonadotrophin was not present in the urine, blood, milk, faeces, or saliva of the cow at any time during pregnancy. Since it has been reported that pregnandiol and oestrogenic substances have been found in the urine of the pregnant cow it was decided to carry out further work in an attempt to determine when these substances first occur in detectable quantities.

PREGNANDIOL

Since the discovery of the corpus luteum hormone, progesterone, attempts made to identify this substance in the urine, the blood and the faeces of women have so far failed.

The isolation of a sterol compound, pregnandiol, from the urine of pregnant women by Marrian (1929) and the establishment of its structural formula by Butendant (1934) led this latter worker to suggest that, as there was a close similarity in structure between this substance and progesterone, pregnandiol might be an excretory end-product of the latter. Venning and Browne (1936) showed that pregnandiol was excreted as a complex with glucuronic acid—sodium pregnandiol glucuronidate—and that it could be extracted quantitatively from the urine of pregnant women. Later, Venning and Browne (1937) came to the conclusion that pregnandiol was a breakdown product of progesterone, that it was excreted during the progestational phase of the menstrual cycle ceasing about four days before the onset of bleeding, and that it was absent from the urine during the first half of the intermenstrual period. Venning (1938) further showed that if pregnancy ensued, the excretion of pregnandiol, which began in the progestational phase, continued and rose steadily throughout pregnancy to reach a maximum in the eighth or ninth month and ceased a few days after parturition. It was demonstrated by Venning, Weil and Browne (1939) that pregnandiol was present in human urine in cases of hypertrophy or neoplasia of the adrenal cortex.

In the purely veterinary field, little work has been published on the excretion of pregnandiol in the urine of the domesticated animals, nor has the possibility been considered of extracting this substance from the urine as an aid to the diagnosis of pregnancy. Marker (1939), in carrying out experiments on the urine of different animals, was able to isolate pregnandiol from both pregnant cows and bulls.

The work reported in the first part of this paper was performed in an attempt to correlate the excretion of pregnandiol in cows' urine, firstly with the existence of a pregnancy, and, secondly, with the duration of time since conception. Further, an attempt was made to correlate the amount of the substance excreted with the histological appearance of the endometrium in slaughtered cows.

MATERIALS AND METHOD

Samples of urine (250 ml.) were taken from the following animals:—

Thirty-four cows at various stages of pregnancy and six non-pregnant cows, from herds in Co. Dublin and Co. Wicklow. (Table 1.)

Eighteen cows at various stages of pregnancy; 16 non-pregnant cows with a corpus luteum in an ovary; six non-pregnant cows with the ovaries in a state of anoestrus, slaughtered in the Dublin Municipal Abattoir. (Table 2.)

Four bulls; four bullocks; one heifer-calf. (Table 3, page 45)

Bulked samples were also taken from slaughtered pregnant cows in an attempt to extract a sufficient quantity of the product to carry out a melting point determination. Bulked samples of urine from pregnant women in the Rotunda Hospital, Dublin, were used in the early part of the work to test the practicability of the method.

For the removal of urinary pigments, the method of Roth, Mayer and Bogart (1941) was used. To extract pregnandiol an adaptation of the methods of Astwood and Jones (1941) and of Guterman (1944) was adopted. The presence of a sterol in the extracted residue was recognised by the fluorescence reaction in which a sterol if heated with concentrated sulphuric acid will give a bright green fluorescence when examined under a high intensity lamp. (Cuboni: 1934, 1935.)

It was also decided to carry out extractions using the methods of Venning (1937) and of Weil (1938). For the extraction of sodium pregnandiol glucuronidate by Venning's method, 1,000 ml. samples of urine were taken from two pregnant cows. An attempt was also made to extract free pregnandiol from like samples of two pregnant cows using the method of Weil.

The weights of the ovaries and of the corpora lutea together with a brief description of the uteri and ovaries of the slaughtered cows (Table 2) were recorded. Sections of the endometrium of

these cows stained with Delafield's haematoxylin and eosin or with Weigart's iron haemotoxylin were also examined.

OBSERVATIONS

The data set out in Tables 1 and 2 show that the quantity of residue extracted from the urine of both pregnant and non-pregnant cows varied from 0·8 mg. to 8·0 mg. per litre. This variation bears no relationship to the presence or the duration of pregnancy, since 1·6 mg./litre was extracted from the urine of a pregnant cow at term (No. 79); a non-pregnant cow (No. 39) yielded 7·2 mg./litre. As the majority of the live cows used in this work belonged to "flying herds," it was rarely possible to obtain several urine samples from the same cow at reasonably wide intervals throughout pregnancy, but in the few cases where this was possible little variation was found in the quantity of residue extracted from each. Three samples were taken from cow No. 25; the first taken at the 97th day of pregnancy yielded 4·8 mg./litre; the second at the 134th day, 4·0 mg./litre; while the third at the 180th day, 4·8 mg./litre. The residue extracted from the urine of six of the slaughtered cows, whose ovaries were inactive, varied from 0·8 to 5·6 mg./litre. The quantity obtained from the urine of four bulls varied from 2·4 to 4·0 mg./litre, while that extracted from three bullocks and a heifer calf was in each case less than 0·1 mg./litre.

In order to carry out a melting point determination, after each experiment the residue was dissolved in absolute alcohol and the solutions bulked together in a flask from which the alcohol could evaporate. A determination was carried out with 10 mg. of residue obtained in this way. It is evident that the substance was impure since it melted at 110° C. and disintegrated at 215° C., whereas the melting point of pregnandiol is 245° C. The residue when heated with concentrated sulphuric acid gave a bright green fluorescence, thus indicating the presence of a sterol. The absence of cholesterol was shown by a negative reaction to the acid anhydride test.

To ensure that the residue contained no oestrogenic substances, 10 mg. of bulked residue was dissolved in 5 ml. ether, to which was added 5 ml. hot olive oil. The ether was evaporated and the oil allowed to cool. The olive oil vehicle containing the residue was injected subcutaneously into an ovariectomised mouse in 1 ml. doses morning and evening for three days, a vaginal smear being taken on the fourth day and stained by Giemsa's method. The cells were of a normal, nucleated type. From this evidence it was concluded that the residue was an impure sterol containing neither cholesterol nor oestrogenic substances.

For the extraction of sodium pregnandiol glucuronidate by Venning's method (1937), like samples were taken from two in-calf cows. The first from a cow 170 days pregnant yielded 4·4 mg. residue, while the second, from a cow 152 days pregnant, gave

5·2 mg. A positive fluorescence reaction for a sterol was obtained in each case, and an emulsion of the residue in olive oil injected into an ovariectomised mouse failed to cornify the vaginal epithelium, thus showing that no oestrogenic substances were present.

TABLE I

EXTRACTION OF PREGNANDIOL FROM COW'S URINE

Number of cow	*Day of pregnancy*	*Weight of residue mg. per litre of urine*	*Result of fluorescence reaction*
1	At term	3·2	Positive
2	At term	7·2	Negative
3	272	4·0	Positive
4	248	6·4	Positive
5	241	5·6	Positive
6	235	4·8	Positive
7	216	4·8	Positive
8	202	2·4	Positive
9	200 (approx.)	2·4	Positive
10	200 (approx.)	4·0	Positive
11	200 (approx.)	1·6	Positive
12	198	3·2	Positive
13	193	3·2	Positive
14	182	4·8	Positive
15	175	2·4	Positive
16	170	4·0	Positive
17	165	4·0	Positive
18	162	5·6	Positive
19 (i)	156	4·8	Positive
(ii)	165	4·0	Positive
20	155	1·6	Positive
21	150 (approx.)	1·6	Positive
22 (i)	150	3·2	Positive
(ii)	162	2·8	Positive
(iii)	163	3·2	Positive
(iv)	166	3·2	Positive
23	140	5·6	Positive
24	104	3·2	Positive
25 (i)	97	4·8	Positive
(ii)	134	4·0	Positive
(iii)	180	4·8	Positive
26 (i)	95	6·4	Negative
(ii)	129	4·0	Positive
(iii)	151	4·0	Positive
27 (i)	92	3·2	Positive
(ii)	120	4·0	Positive
28	89	4·8	Positive
29	89	2·8	Positive
30	80 (approx.)	4·0	Positive
31 (i)	76	4·0	Positive
(ii)	165	4·0	Positive
32	57	3·2	Positive
33 (i)	43	6·4	Negative
(ii)	61	4·0	Positive
(iii)	74	4·0	Positive
34	? 26	4·8	Positive
35	Aborted on previous day	4·0	Positive
36	Not in calf	5·6	Positive
37	,,	3·2	Positive
38	Not in calf (anoestrus)	4·0	Positive
39	,, ,,	7·2	Positive
40	,, ,,	4·0	Positive

TABLE II

EXTRACTION OF PREGNANDIOL FROM URINE OF SLAUGHTERED CROSSBRED COWS

No. of cow	*Description of ovaries*	*Weight of ovaries* grams	*Weight of corpus luteum* grams	*Description of uterus*	*Weight of residue per litre of urine* mg.	*Result of fluorescence reaction*
41	Anoestrus, small follicles and scars	5·32 3·86	—	Anaemic and aplastic	5·6	Positive
42	Corpus luteum, small follicles	9·80 4·77	1·90	Foetus 78 cm.*	2·4	Positive
43	Anoestrus, small follicles and scars	4·96 2·84	—	Anaemic and aplastic	0·8	Positive
44	Corpus luteum, small follicles	10·55 5·69	3·76	Hyperaemic, regressing cotyledons	4·8	Positive
45	Anoestrus, small follicles and scars	7·65 4·42	—	Anaemic and aplastic	3·2	Positive
46	Corpus luteum, small follicles	10·05 4·34	4·20	Hyperaemic, metritis	4·0	Positive
47	„ „	10·93 6·24	3·47	Hyperaemic, regressing cotyledons	4·0	Positive
48	„ „	13·53 8·86	4·07	Foetus 12 cm.*	6·4	Positive
49	„ „	7·49 3·26	3·73	Foetus 3 cm.*	4·8	Positive
50	„ „	11·43 4·62	4·56	Metritis	6·4	Positive
51	„ „	6·40 4·25	3·59	Foetus 25 cm.*	3·2	Positive
52	„ „	11·78 10·02	4·92	Foetus 11 cm.*	4·0	Positive
53	„ „	10·84 3·76	2·73	Hyperaemic, regressing cotyledons	3·2	Positive
54	Two corpora lutea in right ovary, large follicles	12·74 5·65	3·45 2·10	Metritis	1·6	Positive
55	Corpus luteum, large and small follicles	9·80 8·61	3·24	Hyperaemic, metritis	7·2	Positive
56	Anoestrus, small follicles	5·89 4·32	—	Anaemic and aplastic	0·8	Positive
57	„ „	10·75 6·62	—	„ „	2·4	Positive
58	Corpus luteum, small follicles	10·13 2·88	3·80	Foetus 7 cm.*	4·0	Positive
59	„ „	8·05 4·80	3·65	Foetus 25 cm.*	4·0	Positive
60	„ „	8·95 4·96	1·82	Foetus 76 cm.*	3·2	Positive
61	„ „	9·42 4·70	1·93	Foetus 84 cm.*	2·4	Positive
62	„ „	8·62 3·50	3·70	Metritis, regressing cotyledons	4·8	Positive
63	„ „	11·13 7·22	5·12	Foetus 21 cm.*	4·0	Positive
64	„ „	14·20 7·45	5·34	Foetus 20 cm.*	4·0	Positive
65	„ „	9·20 4·23	2·50	Foetus 50 cm.*	5·6	Positive

* All foetal measurements are from crown to rump.

TABLE II—*continued*

EXTRACTION OF PREGNANDIOL FROM URINE OF SLAUGHTERED CROSSBRED COWS

No. of cow	*Description of ovaries*	*Weight of ovaries* gram	*Weight of corpus luteum* gram	*Description of uterus*	*Weight of residue per litre of urine* mg.	*Result of fluorescence reaction*
66	„ „	8·64 4·77	2·23	Foetus 74 cm.*	2·4	Positive
67	„ „	11·04 4·80	3·62	Foetus 8 cm.*	4·0	Positive
68	„ „	9·00 5·61	4·08	Metritis	4·8	Positive
69	„ „	12·22 7·30	4·92	Foetus 28 cm.*	4·0	Positive
70	„ „	10·97 4·58	3·63	Foetus 11 cm.*	4·0	Positive
71	„ „	11·50 5·12	4·83	Metritis, regressing cotyledons	2·4	Positive
72	Right ovary: small corpus luteum, no follicles. Left ovary: small follicles	4·34 2·70	0·82	Metritis, salpingitis right side	1·6	Positive
73	Anoestrus, small follicles and scars	8·12 5·56	—	Anaemic and aplastic	0·8	Positive
74	Right ovary: cystic, large corpus luteum. Left ovary: small follicles	15·14 5·42	7·81	Metritis	5·6	Positive
75	Corpus luteum, small follicles	9·33 5·13	2·45	Hyperaemic, regressing cotyledons	3·2	Positive
76	„ „	8·87 5·04	2·81	Foetus 68 cm.*	3·2	Positive
77	Left ovary: small follicles. Right ovary: cystic; about the size of a pullet's egg. Small corpus luteum	32·50 8·62	0·43	Hyperaemic, regressing cotyledons	2·4	Positive
78	Corpus luteum, small follicles	9·20 5·81	3·97	Hyperaemic	5·6	Positive
79	„ „	9·47 5·09	1·96	Foetus 76 cm.*	1·6	Positive
80	„ „	10·60 4·14	3·10	Regressing cotyledons	3·2	Positive

* All foetal measurements are from crown to rump.

Weil's method (1938) was used with like samples of urine from two cows at term. In neither case was there any precipitate in the alkaline acetone even after chilling in a refrigerator for a week. A similar result was obtained with a bulked sample of urine from pregnant women at term.

In the case of slaughtered cows, the quantity of residue extracted from a sample could not be correlated either with the

histological appearance of the endometrium or with the weight of the corpus luteum. It is an interesting observation that of 40 slaughtered cows whose ovaries were examined the right ovary was in every case heavier than the left, and that where a corpus luteum was present it was contained in the right ovary.

DISCUSSION

The first fact which emerges from these findings is the very small amount of residue which can be extracted from the urine of the in-calf cow. This is in agreement with Marker (1939), who was able to isolate only 43 mg. from a gallon of bulked urine of pregnant cows (9·45 mg./litre). Cope (1940) stressed that in women the significant figure in the excretion of pregnandiol was not the concentration in a single sample of urine, but the total excretion of the substance over a twenty-four hour period. On the other hand, Guterman (1944) based the accuracy of his human pregnancy test on the amount excreted in a 100 ml. morning sample of urine—about one-tenth of the total daily output. A cow excretes about 14 litres of urine during the 24 hour period, and the samples used in this work represent only one fifty-sixth part of the total daily output.

From the data in Tables 1 and 2 it is apparent that the amount of residue extracted from a sample of urine is in no way related to pregnancy or to the presence of a functioning corpus luteum in an ovary; in addition, no relationship could be detected between the quantity of residue extracted and the time of gestation at which the sample was taken. In the case of 34 of the slaughtered cows, it was not possible to correlate the quantity of residue extracted with the weight of the corpus luteum. The writer was unable to find any correlation between the degree of change in the endometrium and the amount of residue extracted from a sample of urine. In this connection two factors must be taken into account, firstly that the residue has been extracted from a sample of urine which represents only one fifty-sixth of the total daily output of a cow, and, secondly, that portions of the same endometrium vary greatly in histological appearance. Since Venning and Browne (1937) found that pregnandiol is excreted in male urine after the injection of sufficiently large doses of progesterone it would seem that an endometrium is not necessary for the conversion of progesterone into pregnandiol. It is assumed that this takes place in the liver.

As small quantities of the residue were extracted from the urine from six anoestrous cows as well as from four bulls, while minute quantities were obtained from three bullocks and a heifer calf, it is suggested that a source of pregnandiol, other than progesterone, exists in the body of the cow. Cuyler, Ashley and Hamblem (1940) demonstrated that pregnandiol was excreted in male human urine after the administration of desoxycorticosterone, a

substance secreted by the adrenal cortex and closely related in chemical structure to progesterone. Thus it would seem that pregnandiol is excreted in the urine as a metabolic product of both progesterone and desoxycorticosterone, which explains the appearance of the sterol in specimens of urine from the anoestrous cows, the bulls, the bullocks, and the heifer calf. It is evident, therefore, that the detection of pregnandiol in the urine cannot be used as a means for the diagnosis of pregnancy in the cow.

OESTROGENIC SUBSTANCES

Both biological and chemical tests are of value for the diagnosis of pregnancy in the mare in which animal comparatively large amounts of oestrogenic substances appear in the urine from about the 120th day. In the in-calf cow, however, the amount excreted is, by comparison, small, and in many individuals they do not appear in detectable quantities until after the fifth month.

Anderson (1934) compared the amounts of oestrogenic substances excreted during pregnancy in the urine of the mare and the cow. He found 200,000 mouse units per litre of urine in the average pregnant mare at term, but only 1,100 units in the cow. From his findings it is clear that at all stages of pregnancy the amounts excreted by the cow are minute as compared with those of the mare. He also found considerable variation in the concentration of oestrogenic substances in the urine of different cows at the same stage of pregnancy. Allen Doisy *et. al.* (1924) described the vaginal smear test which depends upon the capacity of oestrogenic substances to cornify the vaginal epithelium when injected into an ovariectomised mouse. This technique was adopted by Crew, Miller and Anderson (1931) for the diagnosis of pregnancy in the mare. Bruhn (1933) showed that the method might be of use to confirm pregnancy in the cow after the sixteenth week; he was unable to demonstrate oestrogenic substances in cow's urine during oestrus or in pregnancy of less than twelve weeks' duration. On the other hand, Kust (1934) found that the test was valueless in the cow before the 23rd week of pregnancy. Anderson (1934), by concentrating cow's urine, was able to detect oestrogenic substances after the 93rd day, but he found the concentrated urine was very toxic to mice. He considered if non-concentrated urine was used, the Allen-Doisy test was reliable in a cow only after the 150th day of pregnancy. Ocariz and Gilsanz (1934), by injecting ovariectomised rats with acid hydrolysed urine in oil, were able to confirm the presence of oestrogenic substance in cow's urine in 50 per cent. of those examined at the end of the first month of gestation, and in 100 per cent. at the beginning of the fourth month. Skvorcov (1936), using the Allen-Doisy method, was able to demonstrate oestrogenic substances in untreated cow's urine in 77 per cent. of cases after the 100th day of pregnancy and in 100 per cent. after the 250th day. He found that the urine of all cows infected with brucellosis gave positive results whether they were pregnant or not.

A chemical test for the diagnosis of pregnancy in the mare, based on the fluorescence test for sterols, was described by Cuboni (1934, 1935). It has been used with cow's urine for the diagnosis of pregnancy by many

workers—notably Busch (1936), Josef (1936), Frullini (1936), and Dickensheid (1939)—all of whom considered that the reaction was unreliable. On the other hand, Csiki (1942) regarded the test as reliable in the case of buffalo cows pregnant over 100 days.

The work described in this part of the paper was done to confirm or disprove the practicability of the Allen-Doisy test and of the Cuboni test for the presence of oestrogenic substances in the urine of the in-calf cow.

MATERIALS, METHOD AND OBSERVATIONS

For the biological test (Allen, Doisy *et. al.*, 1924) 5 ml. samples of urine were taken from 28 pregnant and five non-pregnant cows. Those obtained from cows later than 140 days after service gave uniformly positive results, while those taken from cows earlier than 97 days after service were uniformly negative. The earliest positive result was from a cow 104 days in calf, while the latest negative result was from an animal 134 days in calf. In every case urine of non-pregnant cows gave negative reactions, including specimens from two non-pregnant cows which were positive to the Cuboni test.

For the chemical test (Cuboni, 1934, 1935) 30 ml. samples were taken from 13 pregnant and five non-pregnant cows. A bright green fluorescence from the heated sulphuric acid-residue solution in a test tube was regarded as positive. Whilst every specimen examined gave a fluorescence, the colour in many instances was a muddy brownish green. These reactions were taken as non-specific and the specimens classified as " negative."

Two specimens from non-pregnant cows (Nos. 37 and 62) gave positive reactions. Another specimen from cow No. 37, taken 14 days after the first, also gave a positive re-action. Samples from the three remaining non-pregnant and from all cows pregnant less than 129 days were classified " negative " on account of a non-specific fluorescence. Samples from three cows pregnant over 180 days were also " negative " for the same reason.

DISCUSSION

There appears to be considerable variation in the excretion of oestrogenic substances in the urine of cows during the first 150 days of pregnancy, as indicated by the Allen-Doisy test. Two of the slaughtered cows (Nos. 51, 59) were each carrying a foetus measuring 25 cm. from crown to rump, *i.e.*, about 120 days pregnant. The urine of the first was negative, while that of the second was positive. Further, cow No. 25 was negative at the 134th day of pregnancy, while No. 24 was positive at the 104th day. From the 140th day onwards all the cows excreted sufficient oestrogenic substance to enable a positive diagnosis to be made. Of the five non-pregnant cows whose urine was tested by this method none gave a false positive reaction. In fact, as already stated, negative

results were obtained in the cases of two non-pregnant cows whose urines were positive to the Cuboni test. The chief disadvantage of the Allen-Doisy technique is that after a mouse has been used for two or three tests the vaginal epithelium seldom returns to its normal condition even after a prolonged rest.

From these findings it would seem that the Allen-Doisy test for the detection of oestrogenic substances in the urine of the in-calf cow is not reliable until after the 140th day of pregnancy. The amounts excreated during the first 140 days of pregnancy are very variable, and in most cases are so small that from a practical point of view a biological test will only rarely enable a diagnosis of pregnancy to be made any earlier than would a clinical examination of the cow.

On the results obtained in this work the Cuboni test is not reliable.

TABLE III

EXTRACTION OF PREGNANDIOL FROM URINE OF OTHER CATTLE

	Weight of residue mg. per litre of urine	*Result of fluorescence reaction*
Four-year-old Hereford bull	3·2	Positive
Four-year-old dairy Shorthorn bull ...	3·2	Positive
Three-year-old Jersey bull	2·4	Positive
Yearling Hereford bull	1·0	Positive
Crosbred bullock	—	Positive
„ „	Less than 0·1	Positive
„ „	Less than 0·1	Positive
„ „	Less than 0·1	Positive
Heifer calf about ten weeks old	Less than 0·1	Positive

SUMMARY

Samples of urine from 72 cows, four bulls, three bullocks, and a heifer calf were examined. Attempts to extract pregnandiol yielded small quantities of impure sterol containing neither cholesterol nor oestrogenic substances.

In the case of the cow, the weighed quantities of the residue varied from 0·8 mg. to 8·0 mg. per litre of urine. The quantity extracted from a sample gave no indication as to whether the cow was pregnant or not, nor, in the case of a pregnant cow, did it indicate the duration of pregnancy.

The observation that small quantities of residue occurred in the urines of six anoestrous cows and in four bulls, while minute amounts (less than 0·1 mg. per litre) were present in three bullocks and a heifer calf, would suggest that a source of pregnandiol, other than progesterone elaborated by the corpus luteum, exists in the body of the cow. The findings of previous workers point to desoxycorticosterone from the adrenal cortex as a second source.

In slaughtered cows, the amount of residue extracted from a sample of urine could not be correlated with the histological appearance of the endometrium, nor was there any evident relationship between the amount of residue extracted and the weight of the corpus luteum.

The results of Allen-Doisy tests carried out on urine from 28 pregnant and five non-pregnant cows, and of modified Cuboni tests on urine from 13 pregnant cows, showed that the Allen-Doisy test can only be regarded as reliable after the 140th day of pregnancy: the Cuboni test is not reliable in any respect.

REFERENCES

Allen, E., Doisy, E. A., *et al.* (1924). *Amer. J. Anat.*, **34,** 133.
Anderson, J. (1934). *Vet. J.*, **90,** 295.
Asdell, S. A., and Madsen, L. L. (1933). *Cornell Vet.*, **23,** 59.
Astwood, E. B., and Jones, G. E. S. (1941). *J. biol. Chem.*, **137,** 397.
Bruhn, G. (1933). *Inaug. Diss. Hannover.* (*Vet. Bull.*, **4,** 782.)
Busch, G. (1936). *Tierärztl. Rdsch.*, **42,** 780. (*Vet. Bull.*, **7,** 477.)
Butendant, A. (1934). *Ber. dtsch. Chem. Ges.*, **67,** 1901.
Cope, C. L. (1940). *Brit. med. J.*, **2,** 545.
Crew, F. A. E., Miller, W. C., and Anderson, J. (1931). *Vet. J.* **87,** 450.
Csiki, V. (1943). *Jber. vet. Med.*, **70,** 543. (*Anim. Breed. Abstr.*, **12,** 193).
Cuboni, E. (1934). *Clin. Vet. Milano,* **57,** 85; (1935). *Ibid.*, **58,** 713.
Cuyler, W. K., Ashley, C., and Hamblem, E. C. (1940). *Endocrinology,* **27,** 172.
Dickenshied, S. (1939). *Geburtsh. Klin. Univ. Berl.*, (*Anim. Breed. Abstr.*, **8,** 127.)
Frullini, P. L. (1936). *Boll. sez. ital. Soc. int. Microbiol.*, **8,** 37.
Guterman, H. S. (1944). *J. clin. Endocrinol.*, **4,** 262.
Josef, A. (1936). *Vet. Med. Dissertation. Univ., Zürich* (*Anim. Breed. Abstr.* **6,** 292.)
Kust, D. (1934). *Berl. tierärztl. Wschr.*, **50,** 734.
Marker, R. E. (1939). *J. Amer. chem. Soc.*, **61,** 944.
Marrian, G. F. (1929). *Biochem. J.*, **23,** 1090.
Ocariz, J., and Gilsanz, L. (1934). *Trab. Inst. Biol. Anim. Madr.*, **2,** 236. (*Anim. Breed. Abstr.*, **5,** 23.)
Roth, S. Y., Mayer, D. T., and Bogart, R. (1941). *Amer. J. vet. Res.*, **2,** 436.
Skvorcov, V. A. (1936). *Trud. Gos. vet. Inst., Vologda,* **1,** 63. (*Anim. Breed. Abstr.*, **5,** 143.)
Venning, E. H. (1937). *J. biol. Chem.*, **119,** 473. (1938). *Ibid.*, **126,** 595.
Venning, E. H., and Browne, J. S. L, (1936), *Proc. Soc. exp. Biol., N.Y.*, **34,** 792; (1937). *Endocrinology,* **21,** 711.
Venning, E. H., Weil, P. G., and Browne, J. S. L. (1939). *J. biol. Chem.*, **128,** cvii.
Weil, P. G. (1938). *Proc. Soc. exp. Biol., N.Y.*, **38,** 503.

[*Received for publication August 15th, 1946*]

A *SALMONELLA* STRAIN ISOLATED FROM CAMELS IN PALESTINE

By

L. Olitzki and V. Ellenbogen,

Department of Hygiene and Bacteriology, The Hebrew University, Jerusalem

INTRODUCTION

In October, 1939, the organs of a camel suffering from enteritis and cachexia were subjected to a bacteriological examination. A bacterium was isolated from the ileum, kidneys, and heart which fermented glucose, maltose, mannose and arabinose with the production of acid and gas but did not ferment lactose and sucrose. Tartrate and citrate were attacked and hydrogen sulphide was formed on Kligler medium. The organism was agglutinated by *S. newport* serum (O) and by *S. typhi murium* serum (H). This strain, together with another isolated from the intestines of a second camel suffering from the same symptoms, were sent to Prof. Klopstock who established their serological identity with *S. kentucky* (antigenic formula VIII XX: i-z6-.)

SEROLOGICAL EXPERIMENTS

The complete identity of the O-antigens of the camel strains with those of *S. kentucky* was shown as follows. Two sera were prepared by the immunization of rabbits with bacteria heated at 100° C. for two hours. Absorption of the *S. kentucky* serum (O) as well as of the camel-strain serum (O) with *S. newport* left agglutinins for both the Kentucky and the camel strains.

Absorption of these sera with *S. kentucky* or with one of the camel strains removed the somatic agglutinins for all camel strains and for *S. kentucky*.

Similar experiments were made with two sera prepared by the immunisation of rabbits with suspensions heated at 60° C. for 30 minutes. The somatic agglutinins were removed by absorption with organisms heated at 100° C., and the following experiments undertaken with the flagellar agglutinins. Absorption of the camel-strain serum with *S. kentucky* removed the agglutinins for all strains of the group; absorption of the *S. kentucky* serum with most of the camel strains removed the H-agglutinins for all the camel strains as well as for *S. kentucky*. One of the camel strains left a well-defined residue of H-agglutinins in the *S. kentucky* as well as in the camel-strain serum but freed the serum of all its own agglutinins. The agglutination of this strain by a specific *S. typhi murium* serum, containing the i-agglutinin, and by a *S. kentucky* serum absorbed with S. typhi-murium and containing the z6-factor showed that it contained only the i-factor representing the *alpha*-phase of the organism as described by Edwards (1938).

The results of an examination of a further group of camels. (Table I) showed that the serum of healthy as well as of infected animals contains O- and sometimes H- and O-agglutinins for the camel strains and for *S. kentucky*. The results with the camel strains as well as with *S. kentucky* were quite uniform. Absorption tests showed that both *S. kentucky* and the camel strains remove the agglutinins for all the strains of the group.

TABLE I

RESULTS OF THE EXAMINATION OF 19 CAMELS FROM DIFFERENT AREAS OF PALESTINE

No.	*Place*	*Age*	*Findings in camel*	*Bacteriological examination*	*Titres of agglutination with the camel strains and with S. kentucky*
1	Transjordania	More than 12 years	Normal	Negative	1 : 100 (O)
2	Gaza	More than 12 years	Cachexia	„	1 : 100 (O)
3	„	More than 12 years	Normal	„	1 : 200 (H and O)
4	Hebron	6 years	„	„	1 : 50 (O)
5	„	10 years	Cachexia, 5-liter peritoneal fluid	„	1 : 50 (O)
6	„	3 years	Normal	„	1 : 50 (O)
7	„	8 years	8-liter peritoneal fluid; diarrhoea; echinococcus in liver and lungs	„	1 : 10 (O)
8	„	7 years	Normal	„	1 : 50 (H and O)
9	„	10 years	„	„	1 : 50 (O)
10	Lydda	12 years	„	„	1 : 50 (O)
11	„	10 years	„	„	1 : 200 (H and O) 1 : 500 — 1 : 1,000 (H)
12	Gaza	6 years	„	„	1 : 100 (H and O)
13	„	8 years	„	„	1 : 100 (H and O)
14	Hebron	8 years	Cachexia, 5-liter peritoneal fluid	*Salmonella* (camel strain) in blood culture	1 : 500 (H and O)
15	„	8 years	Cachexia; diarrhoea; 6-liter peritoneal fluid	Negative	1 : 100 (O)
16	Lydda	More than 12 years	Normal	„	1 : 200 (O)
17	„	10 years	„	„	1 : 200 (H and O)
18	„	12 years	„	„	1 : 500 (H and O)
19	„	More than 12 years	„	„	1 : 200 (H and O)

The strain isolated from the heart blood of camel No. 14 was serologically identical with *S. kentucky*, and with those previously isolated. Of the five animals showing cachexia and enteric symptoms four were from Hebron and one from Gaza. Up to the present no cases have been reported from other parts of the country.

Table I also shows that sick camels have a relatively low agglutination titre. From five cachectic animals only one serum with a titre of 1:200 and more, two of 1:100, one of 1:50 and one 1:10 were obtained. The camel strain was isolated from one of these cachectic camels. On the other hand, the serum from most of the normal camels showed agglutination titres of 1:100 and more. Four camel sera showing the presence of H- and O-agglutinins for *S. kentucky* were tested with six other *Salmonella* strains which have no common H-antigens with *S. kentucky* and the camel strains. The results are given in Table II.

TABLE II

AGGLUTINATION TITRES OF 4 CAMEL SERA FOR VARIOUS *Salmonella* STRAINS

Strain	*Serum No.* 11	*Serum No.* 14	*Serum No.* 18	*Serum No.* 19
S. kentucky	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)	1 : 200 (H and O)
Camel strain	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)
S. paratyphi A	Negative (at 1 : 50)	Negative	Negative	Negative
S. paratyphi B	1 : 100 (O)	1 : 200 (O)	1 : 200 (O)	1 : 100 (O)
S. paratyphi C	1 : 200 (O)	1 : 100 (O)	1 : 100 (O)	1 : 100 (O)
S. enteritidis	Negative	Negative	1 : 50 (O)	1 : 50 (O)
S. typhi (H901)	„	1 : 100 (O)	1 : 200 (O)	1 : 100 (O)
S. london	„	Negative	1 : 50 (O)	1 : 50 (O)

EXPERIMENTS IN MICE

The pathogenicity of the camel strains and of *S. kentucky* was tested by the oral infection of 10 mice which were subsequently observed for a period of 20 days. The results were recorded by noting the specific mortality, based on cultures from the dead mice, and the residual infection as determined by culturing the heart, liver, spleen, kidneys, and mesenteric glands of the surviving mice at the end of each experiment.

The administration of 1,000 million organisms produced the following results:—

Camel strain: (10 mice). One died showing a positive blood culture. Nine survivors were killed; all the organs and the mesenteric glands were sterile.

S. kentucky: (10 mice). No deaths. In the survivors, two mice showed infections of liver and spleen, three infections of the mesenteric glands and five were sterile.

Another group of mice received the same dose on each of two successive days, *i.e.*, a total of 2,000 million bacteria. The results were as follows:—

Camel strain: (8 mice). No deaths. In the survivors two mice showed infections of the mesenteric glands and six mice were sterile.

S. kentucky: (8 mice) No deaths. In the survivors one mouse showed a general septicemia, three showed infections of the mesenteric glands and four were sterile.

A third group received the same dose on four consecutive days with the following results:—

Camel strain: No deaths. In the survivors one mouse showed a general septicemia, one an infection of the abdominal organs, and six mice were sterile.

S. kentucky: Three deaths with positive heart blood cultures. In the survivors one mouse showed an infection of the abdominal organs, one had infected mesenteric glands and three were sterile.

These experiments show that both the camel strain and *S. kentucky* are not very pathogenic for mice; moreover, the mortality is low and the infection is either localised mainly to the mesenteric glands or the animal is sterile. It would seem, however, that *S. kentucky* has a tendency to persist for a longer period in the liver, spleen and mesenteric glands.

The general results of these three experiments were as follows:—

	Camel strain	*S. kentucky*
Total infected	26	26
Died	1	3
Survived:		
Septicemia	1	1
Abdominal organs infected	1	3
Localised infection of mesenteric glands	2	7
Sterile	21	12

The strains isolated from the mice showed in the first generation a partial or total loss of the H-agglutinins. They appeared as O-forms with a total lack of the H-antigens, or they contained only i-antigen representing the *alpha*-phase of the strain. After two or three passages they again showed full agglutinability for the i- and for the z6-agglutinin.

Experiments were next undertaken with strains isolated from infected mice. After feeding 4,000 million bacteria the results were as follows:—

	Camel strain	*S. kentucky*
Total infected	14	14
Dead	0	0
Septicemia	0	0
Abdominal organs infected	3	1
Localised infection of mesenteric glands	3	2
Sterile	8	11

The camel strain after passage through mice showed a somewhat higher number of residual infections than formerly, but there was no mortality.

BIOCHEMICAL REACTIONS

Both *S. kentucky* and the camel strains fermented the following carbohydrates: arabinose, xylose, fructose, glucose, galatose, maltose mannose, melibiose, dulcitol, sorbitol, trehalose; they did not attack sucrose, lactose, melizitose, raffinose, alpha-methylglycoside, amylum, inulin, glycogen.

S. kentucky fermented glycerol and inositol in plain broth, but the camel strains did not attack them.

SUMMARY

A *Salmonella* strain, which showed the serological structure of *S. kentucky* but did not attack glycerol and inositol in plain broth, was isolated from the blood and organs of three camels showing enteritis and cachexia.

The sera from 19 camels were examined; nine contained H- and O-agglutinins for *S. kentucky* and the camel strain, while the remaining 10 showed only O-agglutinis. Four camel sera, which contained H- and O-agglutinins for *S. kentucky* and the camel strains which do not share a common H-antigen with *S. kentucky*, contained only O-agglutinins for other *Salmonella* strains.

With both strains the mortality in mice infected by the mouth was low, but residual infections of the abdominal organs and the mesenteric glands varying from about 20 to 40 per cent. of the infected animals were found three weeks after infection.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help obtained from Prof. A. Klopstock in Tel-Aviv (Salmonella-Centre for Palestine) for the serological identification of the camel strains with *S. kentucky* and for sending the original *Kentucky* strain provided by the International Salmonella Centre (Dr. F. Kauffmann, Copenhagen).

REFERENCES

Edwards, P. R. (1938). *J. Hyg.*, **38**, 306.

[*Received for publication, September 23rd, 1946.*]

TRACE ELEMENT DEFICIENCY IN LIVESTOCK IN THE HEBRIDES

By

George F. Boddie,

Royal (Dick) Veterinary College, Edinburgh.

INTRODUCTION

There is an established tradition in certain districts in the Hebrides that stock cannot be kept in healthy condition if grazed continuously on particular areas. In these districts the removal of stock at regular intervals to non-affected areas has long been practised. Tradition and written record agree that for many years a heavy mortality has occurred amongst cattle, especially young animals and sheep, the losses being greatest during the late winter. In addition to mortality, young stock showed marked retardation of growth which may be so pronounced that an affected animal when eighteen months old is no bigger than a normal one of three months. In some instances there is a definite shortage of winter keep, and in these starvation is probably the chief factor with, in many cases, helminthiasis as a contributory cause. In other young stock, however, although food appears to be adequate in amount and the helminth burden is low, development and growth is seriously retarded; in these cases the winter mortality in animals of all ages is very high. It was readily apparent that some connection existed between the retardation of growth and grazing of young stock on machair or sandy areas. Winter feeding consists almost entirely of meadow hay and oats grown on the sandy soil. The older animals spend the summer on common grazing which is usually on the higher peat soil; they return to the townships for the winter months. Young stock are kept near the township to graze the lower ground and in affected areas the soil in these parts is largely composed of white sand.

The term "pining" has been used to describe the debility which results from trace element deficiency, and there has been a tendency to connect the word with anaemia. It would appear, however, that any anaemia that develops in trace element deficiencies is secondary to debility and inanition. Furthermore, "pining" has been widely used to describe debilitating conditions in cattle and sheep due to such diverse causes as starvation, helminthiasis, trace element deficiencies and specific bacterial disease.

DESCRIPTION OF AREAS INVOLVED

Barra Area. (*Map* 1). *Island of Vatersay.* The soil of the low-lying central area contains a very high proportion of shell sand. When in the latter part of the nineteenth century the island was converted into crofts, this sandy area was chosen for their establishment largely, it appears, because of its position. *Eoligarry.* On the northern side of the hill, Ben More Eoligarry, there is an area about half-a-mile wide which slopes

MAP 1.

gently downwards from the foot of the hill to the coast. It was divided into crofts in 1919. In part of the area the soil is black and peaty, but on the north-eastern fringe it is white and sandy.

South Uist. (*Map* 2). A strip of machair land extends almost the whole length of the west side of the island. *Bornish* is a typical area with a combination of machair and hill ground. *Ormaclett and Dremisdale.* In addition to the grazing area, the land on which hay and corn are grown for winter feed, though higher than the main machair area, consists largely of sand.

North Uist. (*Map* 3). *Baleshare.* This is a tidal island, normally accessible by a track at low tide, the greater part of which is sandy. *Kirkibost.* This also is a tidal island lying to the west of North Uist. It is entirely sandy, the sand being very white. The only water available is collected from surface drainage into a deep ditch, a dam preventing the sea flowing into it at high tide. There is only one farm. *Kyle Paible* is a promontory on the west side of North Uist; its soil is entirely sand. *Vallay.* This is a tidal island lying off the north-west corner of North Uist. Many years ago water was piped to it from the main island, but of recent years this supply has ceased due to corrosion of the pipes. There is a tradition on this island that when cattle had access to water from the mainland they did not become debilitated even when kept on the island for a long time, but adequate corroboration of this could not be obtained.

CLINICAL MANIFESTATIONS

Throughout the areas described, the general clinical picture of the disease is remarkably uniform. Animals grazing solely on machair ground or fed with crops grown on such soil lose their appetite and progressive debility ensues, but anaemia occurs only in those which are emaciated. In young animals growth is markedly retarded. Animals which have regular access to the grazing on black peaty soil thrive well. The general practice is to keep the

younger cattle on the crofts which are usually situated on machair land, while the older cattle are sent to hill grazings in the summer and, while on them, develop normally. In 1944, the stirks on one

MAP 2.

holding in Bornish became affected with sarcoptic mange. Owing, no doubt, to the lowered resistance, the disease became very extensive and several animals died.

In some areas crofters themselves have come to realise that a

nutritional deficiency exists and have adopted methods of counteracting it. At Ormaclett, for example, a man is engaged by the township to move the cattle daily between the machair land and the higher peat land. On Kirkibost the practice is to drive the cattle at low tide across the strand to the mainland of North Uist, where they can graze and drink. If kept too long on Kirkibost both young and old cattle lose their appetite and become extremely debilitated. The rate of recovery of affected animals when transported to the mainland is remarkable; even when emaciated they rapidly regain their appetite and in a fortnight or so begin to thrive. The last tenant farmer on Vallay also had land on the mainland of North

Map 3.

Uist and he periodically moved the stock from the former to the latter. At Dremisdale the clinical picture is complicated by a high incidence of helminthiasis. In Kyle Paible, extreme debility and high mortality among young cattle are accepted by the crofting community as inevitable.

ANALYSES OF SOIL, HERBAGE AND WATER

The clinical manifestations of the conditions bore a similarity to those described by other writers as being caused by a deficiency of trace elements particularly cobalt. Accordingly an investigation was planned to determine the nature, if any, of a trace element deficiency.

Barra Area. Owing to difficulties of inter-island travel, it was

not possible to obtain material for analysis simultaneously from all the areas described. As the Eoligarry area presented the most clearly defined disease picture, it was decided to collect soil samples from the affected crofts in order to make a preliminary investigation. In August, 1943, samples were taken from three crofts (Nos. 25, 26. and 27), and these were analysed at the Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen. In his report, Dr. A. B. Stewart pointed out that they were really specimens of calcareous sand rather than soil in the strict sense of the word and contained 57 to 65 per cent. of calcium carbonate. To obtain crops from land of this character it is necessary to apply heavy dressings of organic manure, and this is done by the application of dung and seaweed. Nevertheless, the solubility relationships are upset by the gross excess of calcium carbonate, and the determination of the amount of any given element may be of no significance, as it may not be available for plant growth. In the circumstances prevailing, Dr. Stewart expressed the opinion that it was unlikely that dressing the land with a manure containing cobalt would be of the slightest use.

In order to establish that cobalt, even if present, was not available to the plant, it was arranged that samples of herbage should be obtained from these crofts. This was done in July, 1944, and the results of the analyses carried out by the Macaulay Institute are shown in Table I.

TABLE I

PASTURE SAMPLES FROM EOLIGARRY, BARRA

Sample	*Constituents as percentage of oven dry pasture*							*Constituents as parts per million oven dry pasture*			
	Ash	P_2O_2	K_2O	*CaO*	*MgO*	Na_2O	*MnO*	*Co*	*Ni*	*Mo*	*Fe*
No. 25	7·9	0·82	1·8	1·45	0·35	1·02	0·025	0·04	0·33	0·45	170
No. 26	7·5	0·64	2·6	1·27	0·37	0·57	0·028	0·02	0·65	0·58	60
No. 27	8·5	0·79	2·9	1·39	0·39	0·60	0·033	0·02	0·32	0·51	42

The cobalt content of all three samples is low, particularly of 26 and 27, and if the content of cobalt-sufficient pasture is accepted as approximately 0·08 parts per million or more, these three samples must be regarded as being definitely deficient. As would be expected the calcium content of the herbage is fairly high, while the other constituents determined fall within the normal ranges.

Feeding of a Cobalt Supplement. As a complement to these analyses, it was decided to ascertain the effect of feeding a cobalt supplement to the stock, but owing to local circumstances, it was not possible to organise a controlled experiment. Moreover, supervision of the trial was difficult as no veterinary surgeon was resident in Barra and transport facilities from South Uist were irregular. During 1944 a cobalt supplement was given to the calves on Eoligarry crofts 25, 26 and 27, and the crofters were of the opinion that

it resulted in improvement of appetite and development. Since, however, it had been made known to the crofters that the condition was probably due to a deficiency in the herbage, it is possible that the calves gained access to other grazing as well as receiving the cobalt supplement.

South Uist and North Uist. The conditions in the areas described in South Uist and North Uist and of Vatersay closely resemble those of Eoligarry since the soils consist largely of shell sand. Dr. R. M. Craig, Lecturer in Economic Geology, Edinburgh University, kindly compared the areas described with his findings when making a geological investigation of these islands. He found them to consist largely of shell sand, the soil having a calcium carbonate content of 60 ± 10 per cent.

In August, 1945, it was possible to obtain samples of soil and herbage from both affected and non-affected areas in the islands of North and South Uist. These were examined by Dr. R. L. Mitchell at the Macaulay Institute. In order to eliminate the possibility that a copper deficiency might play a part in the causation of the disease, analysis of the herbage was extended to include that element.

The results of the soil analyses are shown in Table II.

TABLE II

SOILS

Sample	*pH.*	% $CaCO_3$	% *Loss of ignition*	*Conc. HCl extract mg./100g. air dry*		*0·5N CH_3COOH mg./100g. air dry*		*0·5N Acetic acid p.p.m.*	
				P_2O_5	K_2O	P_2O_5	K_2O	*Co*	*Ni*
No. 1. Representative hair sample from mac-Bornish, South Uist	7·76	48·73	24·88	80·8	21·77	42·0	6·72	—	—
No. 2. Bornish mac-hair, pure machair soil	7·80	52·23	23·83	72·8	15·71	41·0	7·95	—	—
No. 3. Hill ground, Bornish, South Uist	5·23	—	24·70	—	—	2·2	17·0	0·41	0·55
No. 4. Machair at Ormaclett, South Uist	7·78	41·25	21·85	72·8	6·70	24·0	6·72	—	—
No. 5. Meadow at Ormaclett, South Uist	7·76	24·25	21·38	181·5	31·68	30·0	6·72	—	—
No. 6. North end, Kirkibost Island ...	7·76	23·86	12·12	95·7	37·99	21·0	4·68	—	—
No. 7. North end, Kirkibost Island ...	7·76	23·36	12·97	102·1	47·28	30·0	7·95	—	—
No. 8. Middle, Kirkibost Island ...	7·83	23·45	12·38	100·1	38·26	26·0	5·09	—	—
No. 9. Meadow, S'th. End, Kirkibost Island	7·74	9·38	25·77	142·1	46·38	53·5	8·36	—	—
No. 10. Claddach, Kirkibost, mainland of North Uist ...	5·06	—	44·3	—	—	2·3	20·8	0·51	0·98
No. 11. Claddach, Kirkibost, mainland of North Uist ...	6·09	—	33·7	—	—	1·6	26·3	1·02	0·73

The soil on the Bornish machair has a high calcium carbonate content; the *p*H is also high and an estimation of the cobalt content was not possible. The hill ground, however, is a typically acid peat soil with an adequate cobalt content. At Ormaclett the machair has the same general characteristics as at Bornish, though the calcium content is rather lower; the meadow land, though heavily dressed with seaweed and dung, still has a high *p*H, though the calcium carbonate content is lower than the uncultivated machair. The hill ground at Ormaclett is part of the same area as that used by Bornish township. The samples from the uncultivated land of Kirkibost island are remarkably uniform in their content, the calcium carbonate figures being lower than those from the South Uist areas. The soil of the Kirkibost meadow shows the effect of continued dressing with seaweed and dung. Though the organic content of the soil is higher and the calcium carbonate content lower, the soil still retains its high alkalinity. Claddach Kirkibost is the curative area in the mainland of North Uist immediately opposite Kirkibost island. The two soil samples are typical of acid peat soil and the cobalt content appears adequate. The results of the analyses of the herbage samples are given in Table III, which shows the following

TABLE III

PASTURE

Sample	*Constituents as percentages of oven-dry pasture*							*Constituents as parts per million oven-dry pasture*				
	Ash	P_2O_2	K_2O	*CaO*	*MgO*	Na_2O	*MnO*	*Co*	*Ni*	*Mo*	*Fe*	*Cu*
No. 1. Representative sample from machair Bornish, South Uist ...	9·64	0·786	2·04	2·052	0·661	0·618	0·0067	0·07	0·50	0·73	138	6·5
No. 2. Hill ground, Bornish, South Uist ...	11·05	0·992	3·48	0·462	0·475	0·301	0·0756	0·11	0·66	1·27	222	10·6
No. 3. Machair at Ormaclett, South Uist ...	8·26	0·421	1·50	2·124	0·588	0·474	0·0035	0·05	0·36	0·45	101	5·3
No. 4. Meadow ar Ormaclett, South Uist ...	8·66	0·613	2·48	1·598	0·448	0·520	0·0046	0·06	0·32	0·34	73	6·8
No. 5. North End, Kirkibost Island	9·06	0·655	1·87	2·374	0·500	0·316	0·0047	0·06	0·38	0·29	144	12·0
No. 6. Middle , Kirkbost Island	7·30	0·349	2·52	1·741	0·366	0·521	0·0087	0·07	0·43	0·65	96	6·0
No. 7. Meadow, South End, Kirkibost Island ...	8·89	0·637	2·10	1·426	0·416	0·503	0·0125	0·05	0·14	1·14	62	7·3
No. 8. Claddach, Kirkibost, mainland of North Uist	10·38	0·609	3·75	0·610	0·516	0·648	0·0661	0·40	1·61	5·97	344	12·4
No. 9. Claddach, Kirkibost, mainland of North Uist	11·88	0·631	4·00	1·233	0·667	0·664	0·0225	0·22	0·75	5·28	145	16·0

significant points. Herbage from the Bornish machair is below the critical cobalt figure. whereas herbage from the hill ground is above it. In regard to copper, if the figure four parts per million is accepted as the amount required to avoid copper deficiency, herbage

from both the Bornish machair and hill ground appear to contain sufficient of this element. Turning to Ormaclett, it is found that the herbage from the machair and the meadow show a greater cobalt deficiency than was the case at Bornish, but the copper content is higher than the critical figure. A comparison of the herbage analyses from Kirkibost island and Claddach Kirkibost is interesting. The north end of the island and the central portion near the surface water supply produce a herbage containing respectively 0·06 and 0·07 p.p.m. of cobalt, while that of the meadow, which has for many years been dressed with seaweed and dung, contains only 0·05 p.p.m. The copper figures of 12·0, 6·0 and 7·3 p.p.m. are adequate. The herbage from Claddach Kirkibost, a recognised curative area, has a cobalt content of 0·4 p.p.m., while copper at 12·4 p.p.m. is practically identical with that of the north end of Kirkibost island. It is perhaps significant that the low herbage cobalt in the areas where debility and inanition occur coincides with a high soil *p*H and, with the exception of the Kirkibost meadow, also coincides with a high calcium soil content, whereas the higher herbage cobalt figures are associated wth a lower soil *p*H.

Three samples of water were obtained for analysis; one from the surface supply on Kirkibost island, another from the loch in Claddach Kirkibost, and the third from the reservoir on the mainland of North Uist (that which formerly supplied water to the island of Vallay). None of these was found to contain any demonstrable quantity of cobalt; thus no support is given to the local view that the water on the cobalt-sufficient areas has either curative or prophylactic properties.

DISCUSSION

Greig, Dryerre *et al* (1933) described "Pine" as affecting sheep and young cattle in the island of Tiree. They pointed out that in that island there are very large areas of blown sand which have a very high content of shell fragments. It was when grazing on these areas that the animals were affected. The symptoms described by these authors were "progressive debility, accompanied by anaemia and emaciation." In the Tiree experiments the administration of crude ferric oxide appeared to have a specific curative effect. Greig, Dryerre *et al* (1938) described "Border Pine" occurring among sheep in the Cheviots and adduced experimental evidence that the condition could be prevented and cured by the administration of iron and ammonium citrate. Corner and Smith (1938) pointed out that crude ferric oxide contained cobalt to the extent of 50 parts per million, and suggested that the good results obtained from the administration of iron compounds might be due to the presence of cobalt. They described soil and herbage analyses, field tests and controlled experiments with cobalt, that showed that the "pine disease of sheep in the Cheviot region of Scotland can be cured and prevented by the administration of cobalt." Stewart, J., Mitchell

and Stewart, A. B. (1941) investigated "Pining in Sheep" in Rossshire, and showed that it could be cured and prevented by the administration of cobalt. They also showed the value as a preventive of dressing the soil with artificial manure containing sufficient cobalt chloride to provide a dressing of two pounds per acre. Stewart and Holman (1944) state "that cobalt deficiency in itself does not cause anaemia, nor does it materially interfere with haemoglobin formation. Anaemia only becomes apparent as the lambs develop severe debility."

The conditions prevailing in Tiree closely resemble those in the Hebrides. It seems reasonable to assume that the clinical condition in Tiree was due to cobalt deficiency, and that its prevention and cure by the administration of ferric oxide was due to the cobalt present in the latter. In view of the soil chemistry it would appear that the use of a cobalt-containing fertiliser would not be effective in Tiree.

The onset of cobalt-deficiency as a clinical condition in stock in Vatersay and Eoligarry coincided with the division of the land into crofts and the restriction of the area of grazing available to the young animals which were kept on the crofts. The greater freedom of grazing permitted when Vatersay and Eoligarry were conducted as large farms probably prevented the occurrence of the condition. In South Uist and North Uist, large farms have also been divided up into crofts, but crofting has existed in some of the affected areas from time immemorial. The "run-rig" system of land tenure prevailed in the Hebrides until well into the nineteenth century, but appears to have been finally abandoned in 1883. It is doubtful if this system of tenure of the cultivated land in the crofting townships would have had much effect in preventing the occurrence of cobalt-deficiency in animals in the machair. But so long as the "run-rig" system of tenure of the cultivated land prevailed, it was the practice to remove the stock for the summer to the higher inland grazing where they dwelt with the cattle throughout the summer. The calves born in the spring months would be fortified against cobalt deficiency by the period of grazing on the inland hill pastures. As far as can be ascertained, the calving of a cow in the autumn was an unusual and undesired accident.

SUMMARY

A description is given of the clinical syndrome of debility and inanition which occurs among cattle and sheep in certain areas of the Hebrides.

The areas involved are the machair lands in which the soil is composed largely of shell sand. The soil characteristics of these areas are described and the results of micro-analysis of herbage and soil are given and discussed.

The condition described appears to be associated with a cobalt deficiency. Though cobalt may be present in the soil, it is not

available to the plant owing to the very high calcium carbonate content of the soil.

No evidence was adduced to suggest that copper deficiency played a part in the causation of the disease.

ACKNOWLEDGMENTS

I am indebted to Dr. A. B. Stewart and Dr. R. L. Mitchell for the analyses of soil and herbage and for much helpful advice, and to Dr. R. M. Craig for information concerning the geology of the area.

It was largely due to the active co-operation and the help of the Rev. Dominic MacKellaig, Northbay, Barra, that details of the crofts at Eoligarry were available, and the samples of soil herbage obtained.

Mr. M. MacLellan, M.R.C.V.S., Daliburgh, South Uist, has been of much assistance during the course of these enquiries.

REFERENCES

Beveridge, E. (1911). *North Uist.* Edinburgh: W. Brown.

Corner, H. H., and Smith, A. M. (1938). *Biochem. J.*, **32**, 1800.

Greig, J. R., Dryerre, H., Godden, W., Crichton, A., and Ogg, W. G. (1933). *Vet J.*, **89**, 99.

Greig, J. R., Dryerre, H. Corner, H. H., and Smith, A. M. (1938). *Ibid.*, **94**, 335.

Mackenzie Compton. (1936). *The Book of Barra.* Routledge.

Skene, W. F. (1880). *Celtic Scotland,* Vol. III, p. 380. Edinburgh: David Douglas.

Stewart, James, Mitchell, R. L., and Stewart, A. B. (1941). *Empire J. exp. Agric.*, **9**, 145.

Stewart, J., and Holman, H. H. (1944). *J. comp. path,* **54**, 44.

[*Received for publication, September 2nd, 1946*]

LEPTOSPIROSIS IN CATTLE IN PALESTINE

By
S. Freund,
Haifa.

INTRODUCTION

Since 1941, when the occurrence of Icterohaemoglobinuria in cattle in Palestine was first recorded by the author, several outbreaks of the disease involving some hundreds of animals have been experienced. It seems that prior to 1941, when the disease was first noticed in animals of Jewish settlers, an identical condition, called "Safra," occurred not only among native cattle, but also in sheep and goats belonging to the local Arabs.

In 1935 an unknown disease of cattle, which affected young stock mainly, appeared in various districts of the Northern Caucasus. The symptoms were high pyrexia of short duration, blood in the urine, jaundice and sometimes necrosis of the skin. Some animals showed hyperexcitability with attacks of madness, while others exhibited general depression and even paralysis. Three clinical forms could be distinguished; peracute, acute, and chronic with relapses. The *post-mortem* changes were intensive jaundice except in cases occurring after the middle of August when marked pallor only was seen, enlarged soft liver, haemorrhages and grey patches on the surface and in the parenchyma of the kidneys and haemorrhages in the lymph glands and heart. From the blood, liver and spleen of calves which died from the disease Michin and Azinow (1935) isolated a Leptospira which they regarded as the casual agent and named it *L. icterohaemorrhagiae vitulorum* on account of its similarity to Weil's Leptospira. Later, Michin, Azinow and Salikov (1941) recorded the successful transmission of cultures of the organism to guinea-pigs and mice. Ponomarenko, Diesperov and Gorovy (1936) recorded a similar disease of calves in the Ukraine which was confined to animals one to seven months old, and arose between May and August. All the cases occurred at pasture and early recovery was the rule provided they were removed as soon as symptoms developed. The disease usually started with a high temperature of 40 to 42° C, and lasted only till the appearance of blood in the urine. *Post-mortem* examination revealed parenchymatous hepatitis, glomerulonephritis and swelling of the spleen. The authors were unable to establish the presence of leptospiral infection, but in view of the similarity of the disease to cases in the Northern Caucasus they could not dismiss the possibility that it was of this nature. Nikolskii (1937) described a disease of young calves two weeks to eight months old in the Lower Volga districts with symptoms of high temperature, blood in the urine, conjunctivitis, diarrhoea and yellow discoloration of mucous membranes. He failed to find *Leptospira* on bacteriological examination, and considered the disease to be more like that of the Ukraine than that of the Northern Caucasus. Jungherr (1944) described a form of Leptospiral jaundice in America which is probably very similar to, if not identical with, icterohaemoglobinuria in Palestine.

The cause of the disease in Palestine was first ascertained by Bernkopf (1945), who isolated a strain of *Leptospira* from cattle and man, which he called *L. bovis*. He found that it differed in its serological characters and pathogenicity from both *L. icterohaemorrhagiae* and *L. canicola,* was capable of producing the disease experimentally in calves, but appeared to be non-pathogenic for laboratory animals. Its exact relationship to the Russian strain, *L. icterohaemorrhagiae vitulorum,* is uncertain as the latter is infective to laboratory animals and moreover under natural circumstances it attacks young stock only, whereas *L. bovis* shows a preference for adult cows and affects calves only during long and severe outbreaks.

THE DISEASE IN PALESTINE

The account which follows is based on observations of outbreaks among cattle belonging to Jewish settlers. The disease causes serious economic loss to stock and is becoming of increasing importance, although damage to flocks of sheep appears to be negligible. From the time of the first outbreak in 1941 up to November, 1943, about 500 cases have come to the author's knowledge. Most of these have been adult cows, and only one bull has succumbed to the disease.

Epidemiology and Susceptibility.

According to Russian workers the main sources of infection of cattle in the Caucasus are swamps and lakes from which the animals drink water. This cannot apply to the Jewish settlements in Palestine as the cattle there are watered from very deep wells. There is a distinct possiblity that rats are carriers and spreaders of the infection. Direct contact can be excluded as no new purchases had been introduced on to the farms in any of the cases investigated. According to Bernkopf (1946), Casper and Reif (1946) the disease is easily spread to human beings. Similar observations were made by Michin *et al.* (1944), who claimed that human beings may act as spreaders of the disease, as cattle and man are both affected with the same *Leptospira.* They also suggest that rats, mice and mosquitoes may act as vectors.

Cows seem to be most susceptible to *L. bovis,* but goats, sheep and human beings may also contract the disease. In human beings the infection resembles Weil's disease, and veterinary surgeons, butchers and farmers, who come in contact professionally with cattle alive or dead, are most likely to contract it.

Course and Symptoms

Three forms of leptospiral jaundice can be distinguished in Palestine.

Peracute form. This occurs during pregnancy or at the time of parturition, as well as in cows suffering from other acute diseases, *e.g., Piroplasmosis.* The onset is very sudden, the temperature rises rapidly to 42° C, the urine becomes black as ink, and all visible mucous membranes slowly become dark brown (jaundice). Extreme weakness and bradycardia, complete cessation of rumination and

general stasis of the digestive system are pronounced. Albumen (in large amounts), bilirubin, urobilin, urobilinogen and haemoglobin are usually found in the urine, and bilirubin is present in the blood serum. Most cases die within three to seven days.

Acute and subacute forms. These are the commonest manifestations of the disease, and are met with in milking cows. The illness lasts about two weeks, and most of the affected animals recover. The onset is slow, and is usually first noticed by the milker, as the milk from one or more quarters becomes pink in colour and contains blood clots. In some instances the pink discoloration of the milk is the only noticeable symptom; in more acute cases the milk is decreased to a few drops of a dark viscous fluid, and symptoms of jaundice appear followed by brachycardia, cessation of rumination with occasional colicky pains, pyrexia, and stasis of the whole digestive system. After lasting for about two weeks these signs slowly disappear, but convalescence is prolonged, taking up to two months. Rectal examination, even when carried out during the convalescent period, reveals that the kidneys are enlarged to about double their original size ; their return to normal takes places very gradually. Abortion at any period of pregnancy is a constant feature, and in many cases is the first indication of the onset of the disease. In some animals an exanthema, resembling a burn, appears on the teats and usually persists for a long time, even after the animal has recovered. Very young calves infected in the first few days of life usually die, but older calves which contract the disease in the acute or subacute forms exhibit black discoloration of the urine, and very quickly recover. In all acute and subacute cases, whether in mature cows or in calves, haemoglobinuria and a rise of body temperature to about 40° C, or over, are constant symptoms.

Chronic and recurrent forms. The chronic form is very similar to the acute or subacute, but the symptoms are less pronounced and last much longer. In the recurrent form the animal, after apparent recovery from an attack, suffers a recrudescence with all the usual symptoms a few days later. In some outbreaks, abortions are the only sign of the chronic form, and there is no trace of jaundice or blood in the urine. In some byres a single cow may evince typical symptoms of the chronic form, while others show evidence of digestive disturbance only; it is then difficult to decide whether the latter is a sign of the disease or just a coincidence. In the majority of instances, however, the blood serum of such cows has shown a very high agglutinating titre for *L. bovis*.

Treatment

Treatment did not prove to be of great value in any form of the disease. Russian workers have claimed good results from the use of Neosalvarsan, but when Evarsan was tried in Palestine its effects were not convincing. Penicillin has proved to be very successful in the treatment of human cases; the possibility

of using it in the case of valuable animals should, therefore, not be overlooked.

Post-mortem CHANGES

Coagulation of blood is often incomplete. All parts of the body show a dark brown discoloration, less pronounced in milder cases. The liver is greatly enlarged and pale brown in colour with somewhat lighter patches. The spleen is normal or slightly enlarged, and shows no macroscopical changes except that the capsule is often brown in colour. The most striking changes occur in the kidneys, which are usually very dark brown or black in colour and two to three times the normal size.

Histopathological examination reveals focal necrosis of the liver and perilobular infiltration with cellular elements. The kidneys show widespread nephritis affecting the glomeruli and tubules to a more or less equal degree. The lumina of the renal tubules contain products of disintegrated blood giving an iron reaction, and the same products are present in the blood vessels of the kidney. Pigment giving an iron reaction is also present in the spleen.

Ungar (1946) made an histological examination of nine calves infected experimentally with *L. bovis*, all of which died two to three days after the onset of the disease with symptoms of severe jaundice. He found that the liver showed dissociation of cords, cholangitis with retention of bile in the bile ducts, erythrophagia and haemosiderosis in Kupffer cells. The kidneys showed a moderate, non-purulent, interstitial nephritis. Levaditi's method revealed *Leptospirae* sparsely distributed throughout the liver and kidneys. Of four animals which were only slightly ill for a few days without being jaundiced three showed no marked changes in the internal organs when sacrificed about one week after the return of their temperature to normal, while the fourth revealed severe interstitial nephritis with areas of suppuration and very large numbers of *L. bovis* in the renal tubules and interstitial tissue. Ungar considered that the necropsy findings did not warrant a definite conclusion as to whether the jaundice was due to a hepatogenous, or a haemolytic mechanism, although the latter seemed to him the more likely.

SUMMARY

The course and symptoms of icterohaemoglobinuria, a disease of cattle in Palestine caused by *Leptospira bovis*, are described and the gross and histological features of the lesions noted. The organism is distinct from both *L. icterohaemorrhagiae* and *L. canicola*. The mode of infection in cattle is as yet unknown, but human beings always contract it by the handling of diseased animals or carcases.

ACKNOWLEDGMENTS

The author tenders his sincere thanks to Professor S. Adler for the interest he has taken in this work, and to Dr. Bernkopf and Dr. Casper for

their investigation on the aetiology of Icterohaemoglobinuria. He wishes to express his gratitude to all veterinary surgeons who collaborated with him in investigating the disease in Palestine.

REFERENCES

Bernkopf, H. (1946). *Harefuah,* **30,** 109. (Palestine).
Casper, J., and Reif, L. (1946). *Ibid,* **30,** 113.
Freund, S., Trainin, D., and Malkin, M. (1941). *Refuah Veterinarith,* **8,** 153.
Jungherr, E. (1944). *J. Amer. vet. med. Ass.,* **105,** 276.
Michin, N. A., and Azinov, S. A. (1935). *Sovyet Vet.,* **10,** 23.
Michin, N. A., Azinov, S. A., and Salikov. (1941). *Veterinariya* Moscow, **1,** 51.
Michin, N. A., and Leonoff. (1944). *Course of Microbiology* (trans. title) 291.
Nikolskii, M. (1937). *Sovyet, Vet,* **5,** 38.
Ponomarenko, F. M., Diesperov, A. G., and Gorovy, U. N. (1936). *Ibid.,* **5,** 11.
Ungar, H. (1946). *Harefuah,* **30,** 111. (Palestine).

[*Received for publication, October 15th, 1946*]

ORNITHOSIS (PSITTACOSIS) IN A PIGEON FLOCK

By

D. L. Hughes,

The Agricultural Research Council's Field Station, Compton, Newbury, Berkshire

INTRODUCTION

Psittacosis, a disease of birds transmissible to man, has been recognised for over 60 years, and until comparatively recently the range of hosts in birds was thought to be limited to the order Psittaciformes. Parrots, macaws, parrotlets, cockatoos, cockateels, lorikeets and budgerigars have all been incriminated. In fact 31 species in 19 genera within the order Psittaciformes have been found to harbour the infection (Meyer, 1944). It has also been noted that finches and canaries might contract the disease when in contact with infected psittacine birds, and Java rice birds (Padda oryzivora) have been used for experimental studies.

Nevertheless, from the public health point of view the problem appeared to be limited to the prevention of the spread of infection from diseased psittacine birds to man. This view had to be modified when Haagen and Mauer in 1938 demonstrated the presence of psittacosis virus in fulmar petrels (Fulmarus glacialis). Their attention was drawn to the disease in these birds by Rasmussen (1938), a medical practitioner in the Faroe Islands, who had observed many cases of an infectious disease in man which appeared to be associated with the catching and preparing for food of young fulmar petrels. Coles (1940) in South Africa was the first observer to record the occurrence of the disease in the domestic pigeon. His findings were rapidly confirmed in the U.S.A. (Pinkerton and Swank, 1940; Meyer, Eddie and Yanamura, 1942). Andrewes and Mills (1943) first recorded the disease in pigeons in this country.

The present communication records certain features of the disease as it occurred in a self-contained pigeon flock at the Field Station of the Agricultural Research Council. The complete flock was established in November, 1941, by purchasing all the birds from one owner who had imported the foundation stock from America. By January, 1942, dead pigeons and culled birds were being submitted to routine post-mortem examination. During the first half of 1942 the death rate did not appear to be abnormal, and the birds examined *post-mortem* were mostly adults. Some of these pigeons presented pin-point necrotic foci in the liver, but these lesions failed to yield bacteria which could be regarded as the causal agent. There were three cases of fatal haemorrhage from enlarged spleens. In the autumn the proportion of young birds examined *post-mortem* increased, and pericarditis and/or peritonitis became an increasingly common finding.

On October 2nd, 1942, we were informed by Dr. C. H. Andrewes of the National Institute for Medical Research that he had isolated

a virus resembling psittacosis from pigeons supplied from this flock. These birds are described by Andrewes and Mills (1943) in their paper as coming from a farm in Berkshire. By November 7th, 1942, it was possible to confirm that the disease existed in the pigeon flock. The virus was first isolated from an eight-week-old White Carneau cock pigeon, which showed no macroscopic lesions of disease at autopsy. Within the month three more strains of virus had been isolated by means of intra-cerebral inoculations of pigeon tissues into mice. These strains of virus were passaged in mice for five or six generations. Further evidence of the presence of the disease was provided by Lieut.-Colonel J. E. Smadel, of the U.S. Army Medical Corps, who found that blood sera from certain pigeons in the flock fixed complement in the presence of psittacosis antigen.

METHODS OF DIAGNOSIS

In all cases virus was isolated by the use of mice. Portions of pigeon liver and spleen removed asceptically were ground with sterile sand in Griffith's tubes and the material was suspended in 30 per cent. horse serum saline, to make an approximately 10 per cent. suspension. The suspension was lightly centrifuged to throw down the larger particles of tissue and the supernatant was used for the intra-cerebral inoculation of mice, under light ether anaesthesia with 0·03-0·05 ml. The inoculum was tested for bacteriological sterility.

The experimental disease was not characteristic. Most mice had ruffled hair, the eyes were closed and the eyelids frequently stuck together with discharge. Usually the back was arched and the hind legs dragged when the animal tried to move. The strains of virus obtained from this pigeon flock killed mice four to seven days after intra-cerebral inoculation, but failed to cause any obvious disease by intra-peritoneal inoculation. A positive diagnosis was made if the elementary bodies of the virus were detected in impression films from the brain surfaces of infected mice stained by Machiovello's method. The elementary bodies were usually intra-cytoplasmic and were massed either into tight mulberry-like clusters occupying the whole of the cell, or into looser aggregations in which the shape of the individual elementary bodies could be discerned. In some instances the cells had ruptured discharging elementary bodies across the field. With Machiovello's stain the virus particles generally stained predominantly red against the blue cellular background.

In pigeons with pericarditis and peritonitis, elementary bodies could be demonstrated microscopically in smears of the exudate. In these films the picture was somewhat different, for intra-cytoplasmic collections of elementary bodies were neither as common nor as definite as in mice and the common finding showed a wider distribution of individual virus particles throughout the field. Virus was not demonstrated microscopically in pigeons which had no exudative lesions. (Fig. 1.)

SYMPTOMS

It has not been possible to define any typical clinical syndrome in affected birds in this flock. Squabs and squeakers were usually thin, undersized and feeble. Diarrhoea was common and the vent feathers were frequently matted with greyish green concretions. Adults were usually found dead, premonitory symptoms having been absent. In one or two birds, opisthotonus was observed before death.

Post-mortem FINDINGS

Two main types of disease process have been observed.

In the first there was an acute or sub-acute inflammation of the serous membranes, most commonly found in the young birds of the squab and squeaker class, *i.e.*, up to 16 weeks of age. The pericardium and peritoneum were completely covered by a dry fibrinous exudate which caused adhesions between the intestinal coils and the liver. In some instances the visceral organs were matted together and the individual organs could only be separated with difficulty. Inflammation of the peritoneum was of a localised nature in the less acute cases, frequently being limited to the lining of one of the abdominal air sacs. Enlargement of the liver and spleen occurred in some of these birds.

The second form of the disease was more common in older birds and appeared to be associated with a latent virus infection. There was enlargement of the liver, the edges of the organ were rounded and intense vascular congestion was common. In some cases the spleen was enlarged. An increase of over 1 cm. in length was considered significant. The enlargement was sometimes considerable, and spleens 2-3 cm. long by 1 cm. in diameter have been observed. In a few cases this splenomegaly resulted in sudden death caused by subcapsular haemorrhage followed by rupture of the capsule and bleeding into the abdominal cavity. Enlargement of the spleen, however, did not appear to be so constant as in psittacine birds. Involvement of the lungs was not seen, although Coles (1940), in his original description of the disease in pigeons, has micro-photographs of elementary bodies in lung impressions.

Necrotic foci about pin-head size were observed in the livers of some pigeons. In a few birds necrotic pancreatitis was also found varying from complete wedges of necrotic tissue replacing half the normal tissue to one or two pin-point foci. The relationship of these necrotic areas to infection with psittacosis virus is discussed below.

In three instances virus was isolated in mice from pigeons which showed no obvious macroscopic lesions.

Table I summarises the lesions in pigeons from which either virus was obtained by intra-cerebral inoculation of mice with liver and spleen emulsion, or virus particles were seen by direct micro-

scopic examination of smears from inflamed serous membranes in the birds.

TABLE I

Postmortem LESIONS IN RELATION TO AGE GROUP AND METHOD OF DIAGNOSIS

Age group	*Number*	*Pericarditis and/or peritonitis*	*Enlarged spleen*	*Liver lesions*	*Necrosis of pancreas*	*No obvious lesion*	*Diagnosis*	
							By i/cerebral inoc. of mice	*By direct film*
Squab	3	2	2	1	1	—	1	2
Squeaker	27	21	6	14	2	3	6	21
Adult	4	2	2	3	1	—	2	2

HISTOLOGICAL CHANGES

Sections of exudates on serous surfaces, such as the pericardium, revealed intense inflammatory infiltrations with large mononuclear cells and lymphocytes with some polymorphonuclear leucocytes. In sections of pericarditis the inflammatory reaction penetrated the superficial layers of the myocardium. In organs, such as the liver, spleen and kidneys, there was intense vascular congestion and the parenchymatous cells showed changes varying from cloudy swelling to complete necrosis. In the spleen there were frequently areas of haemorrhage. Necrotic foci in the liver were usually pin-head in size, but in the pancreas in certain cases the areas of necrosis were considerable, in one bird involving half the organ.

Smadel, Wall and Gregg (1943) described an outbreak of Psittacosis in a pigeon flock associated with the production of intranuclear inclusion bodies of the acidophilic herpetic type in the liver, spleen and pancreas of six birds examined. The lesions in the pancreas of some of the birds examined by the author closely resembled those described by Smadel and his colleagues and consequently a search was made for similar bodies in tissue sections. Intranuclear bodies were seen in the parenchymatous cells on the edges of necrotic foci in the liver and pancreas. They comprised large acidophilic inclusions occupying most of the nucleus. All that remained of the nucleus was a pale halo surrounding the inclusion and a sharply defined limiting membrane. (Figure 2). One of the sections containing these inclusions was examined by Col. Smadel who expressed the opinion that they were similar in appearance to those described by him.

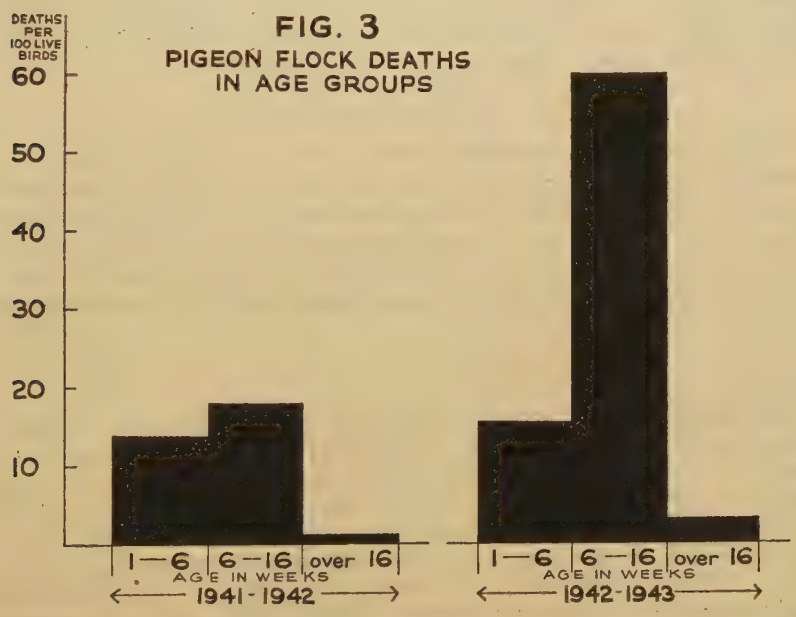
Further work by Smadel, Jackson and Harman (1945) has shown that these intranuclear inclusions represent a virus which is separate and distinct from that of psittacosis. They were able to separate the two viruses, and in one pigeon the intra-nuclear inclusion agent was present by itself. The new agent, which they tentatively called

I.N.I. virus, was non-pathogenic for laboratory animals, but it could be cultivated on the chorio-allantoic membranes of embryonated eggs, and was infective to pigeons. Smadel also found that the serum of a Compton pigeon contained antibodies for the I.N.I. agent. It is probable, therefore, that at least some of the pigeons in the flock were infected with both the Psittacosis virus and the I.N.I. virus. For this reason one would hesitate to ascribe the pathological and histological changes observed as due to psittacosis virus alone.

Other concurrent and secondary infections were not common. On two or three occasions *Salmonella typhi-murium* (*var. Storrs*) was isolated and towards the end of 1945 there were some cases of trichomoniasis, but these organisms were not regarded as serious complications of the primary psittacosis infecton.

DISTRIBUTION OF THE DISEASE WITHIN THE FLOCK

It is the custom at this station for weekly returns to be made of the number of birds in the pigeon flock, giving the total number of birds in each age group, the number of deaths in each age group and the number of birds issued for experimental work. The pigeons,



for the purposes of returns, are divided arbitrarily into the following age classes: 1-6 weeks (squabs), 6-16 weeks (squeakers) and birds over 16 weeks. The death rates from all causes expressed as the average death rate per 100 live birds for the years 1941-42 and 1942-43 are shown diagrammatically in Figure 3. The averages were calculated

from the total deaths in each group in every four-week period. For example in the year 1941-42 in every four week period an average of 17·9 per cent. of the squeakers died.

It will be seen that the mortality was heavy in young pigeons up to 16 weeks of age, and the greatest number of deaths were in the 6-16 week old group. These had left the nests and were no longer being cared for by their parents. The difference in the death rates for the three groups became even more marked during 1942-43, when the mortality in squeakers averaged over 60 dead birds for every 100.

The above figures refer to the mortality from all causes. A more detailed analysis was therefore undertaken to ascertain what proportion of the total deaths were due to psittacosis. The *post-mortem* records for the first 10 months of 1944 were divided into three classes: (1) those with lesions typical of psittacosis; (2) those with lesions which might have been psittacotic in origin; and (3) those that had died from other causes.

The results showed that of 157 pigeons which died, 54 (34·4 per cent.) were affected with psittacosis; 45 of these were birds under 16 weeks old. Lesions which may have been due to psittacosis were found in 21 (13·4 per cent.). It is possible, therefore, that the total deaths due to the virus disease may have been as high as 47 per cent. Deaths from other diseases were 82 (52·2 per cent.).

VACCINATION EXPERIMENT

Little appears to be known about immunity to ornithosis, and although mice can be successfully vaccinated for cross immunity studies of the psittacosis-lymphogranuloma group of viruses, there seems to have been no attempt to protect pigeons against natural infection. It was, therefore, felt worth while to endeavour to confer sufficient immunity to tide the young birds over the susceptible age period.

Methods

A necessary pre-requisite of any virus immunising agent is that it should be rich in virus particles. Smadel *et al* (1943) have described a method for preparing psittacosis antigen for complement fixation tests using the yolk sacs of embryonated chicken eggs as the growth medium. A modification of their method was used for preparing the vaccine.

The ornithosis virus used for the production of vaccine was a pigeon strain, P4E., obtained from Lt. Colonel J. E. Smadel. This strain was well adapted to the yolk sac of chicken embryos and it was kept by passage in them at regular intervals. Between passages the virus material in yolk sacs was stored in containers surrounded by carbon dioxide snow at -70° C.

Five to six day old chicken embryos were inoculated in their yolk sacs with 0·2 ml. of a 1/10 suspension of infected yolk sacs ground up with sterile sand in 30 per cent. horse serum saline. A fresh syringe was employed for every five eggs and the bacteriological sterility of each syringe

was tested after its use. Between three and five dozen eggs were inoculated for each batch of vaccine. The eggs were incubated for 72 hours at 37° C., and then the yolk sacs were harvested with sterile precautions. A representative number of smears of yolk sacs stained by Machiovello's method were examined microscopically for abundance of elementary bodies and freedom from bacteria. The bulked yolk sacs were then placed in a sterile vessel and 7 ml. of a 1/50 dilution of McIlvaine's citric phosphate buffer solution at *p*H 7·6 was added for each yolk sac. This mixture was reduced to a homogeneous suspension by means of a turbo reactor which completely broke up the yolk sac tissue. The whole operation was performed under sterile conditions. A small amount of this suspension was removed, diluted with 30 per cent. horse serum saline in serial ten-fold dilutions and tested for potency by intracerebral inoculation of mice with 0·05 ml. of inoculum. Three mice were used for each dilution. The titre of the vaccine was taken as the highest dilution of inoculum which killed mice within 10 days of inoculation. Death was attributed to ornithosis only if elementary bodies were detected in smears from the brain surface. Immediately after a sample of the yolk sac suspension had been removed for titration formaldehyde was added to 0·3 per cent. This formalinised preparation which constituted the vaccine was stored at 5° C. until it was used. No batch of vaccine was used unless it had a titre of 10^{-5} before formalinisation, and it had satisfied the usual criteria of bacteriological sterility and freedom from live virus after formalinisation.

Pigeons were inoculated at three weeks of age and again three weeks later. The dose was 1 ml. on each occasion, given intramuscularly in the pectoral region. Only alternate pigeons were inoculated. In most cases this procedure resulted in a vaccinated pigeon and a non-vaccinated control bird occupying the same nest.

Vaccinated and control pigeons were kept under observation up to the age of four months, and all birds which died were examined *post-mortem*. After four months of age it was impossible to retain many of the pigeons under observation as they were needed for other work. The results were as follows: vaccinated, 29 ; deaths, 11. Controls, 24 ; deaths, 10.

It will be seen that the deaths in the vaccinated and control groups were virtually the same. It should be pointed out that the deaths referred to are deaths from all causes as it was not always possible to confirm a diagnosis of ornithosis.

However, there were six confirmed ornithosis deaths in the vaccinated birds and five in the control group. The difference in the two groups is not significant, and the only conclusion possible is that the vaccine described above failed to protect pigeons against natural infection with ornithosis virus.

Discussion

As far as the literature has been searched it has not been possible to find an account of Psittacosis in a self contained pigeon flock, in which the observations have continued over an extended period. Once the unit had been set up, the only additions to the population were those resulting from the breeding activities of the adult birds.

The accommodation for the pigeons and their husbandry were such that it is unlikely that extraneous infections were introduced. Therefore, it is a reasonable assumption that such infections as occurred were introduced in a latent form with the original parent stock and that one was dealing with, at least in the case of Psittacosis, an endogenous infection in a closed community. Nevertheless, the community was constantly receiving fresh recruits in the form of nestlings hatched within the flock.

Experience in this pigeon flock has shown that the disease is primarily a disease of young birds. It is of interest to note that the infection did not assume serious proportions in our lofts until the population of young birds had reached a certain level, that is to say, until there were sufficient young birds susceptible to the virus. In the autumn of 1945, it was decided to stop breeding for a period of four months, and coincident with the rise in the average age of the flock there was a decrease in the incidence of the disease. This change in management, however, was also accompanied by other general improvements in husbandry which may well have played a part in the decrease in the death rate.

The evidence in this flock confirms the view that most birds become infected in the squab and squeaker stage and, according to environmental conditions, varying numbers of them succumb to the virus. Of those birds which survive, some may completely recover while others may harbour a latent infection and pass the disease to their nestlings and other young birds in the next generation. Latently infected pigeons may ultimately die from the disease, from such lesions as rupture of an enlarged spleen or chronic inflammation of serous membranes. Latent infections may be brought to light by unfavourable conditions. Thus Pinkerton and Swank (1941) first noted the disease in U.S.A. in pigeons which were thiamine deficient. According to Meyer (1944) up to 10 per cent. of lofted pigeons are found to be latently infected when their sera are tested for complement fixing bodies to the virus.

It would appear that the term Ornithosis which has been adopted by some workers should be used for the virus infecting birds other than the Psittacine class for although on morphological and immunological grounds there appears to be no difference from the Psittacine strains, there is a definite difference in virulence between strains of virus isolated from psittacine birds and those obtained from pigeons. Thus it is not possible to isolate the virus from pigeons by the intra-peritoneal inoculation of mice, although this is the routine procedure for obtaining virus from psittacine birds. Ornithosis virus from pigeons may infect man, and there are records of 17 infections with two deaths (Meyer, Eddie and Yanamura, 1942: Smadel, Wall and Gregg, 1943) directly attributable to contact with diseased pigeons. However, in spite of the widespread distribution of the disease in pigeons the infection in man has not yet become a public health problem as is the case with psittacine birds.

It is of interest that during the four years covered by this communication four different attendants were working in these pigeon lofts and no illness occurred in them which could be attributed to ornithosis virus, although such a possibility was envisaged. Careful precautions were taken against laboratory infections, but it is doubtful if they would have sufficed against classical psittacosis, a disease notorious for causing infection among laboratory workers. Nevertheless, no illness resembling psittacosis developed in the two laboratory workers at risk.

Finally attention must be drawn to the probably widespread distribution of the disease in pigeons. Meyer and his co-workers maintain that the disease is ubiquitous in the U.S.A. Andrewes and Mills (1943) examined pigeons from four separate sources in this country: (1) a London dealer, (2) a farm in Berkshire (those referred to in this article), (3) pigeons recently imported from the U.S.A., and (4) pigeons trapped in Westminster. Ornithosis virus was isolated from each group except the first.

It is obvious that Ornithosis must be regarded as one of the more serious diseases of pigeons, especially where environmental factors are favourable for its development and spread. Infectious disease in these birds must be treated with respect in view of the possible risk to man, and any vague, influenza-like condition in persons in contact with pigeons should immediately bring to mind the possibility of a Psittacosis type of infection.

SUMMARY

An account is given of Ornithosis (Psittacosis) in a self-contained pigeon flock.

The distribution of the infection within the different age groups revealed that the disease primarily affected birds under 16 weeks of age, and suggested that it was maintained by latently infected adult carriers who had survived the disease when young.

There was microscopic evidence that the I.N.I. virus (Smadel *et al.,* 1945) was also present and coexisted with Ornithosis virus.

An attempt to protect young pigeons against natural infection with ornithosis virus by means of a formalinised vaccine prepared from infected chicken embryo yolk sacs failed.

ACKNOWLEDGMENTS

I am indebted to Dr. C. H. Andrewes, of the National Institute for Medical Research, for first drawing my attention to the infection in the pigeons. My thanks are due to the Director, Dr. W. S. Gordon, for his help and direction, and to Mr. F. J. Baker for his technical assistance, and Mr. W. G. MacLeod, of the Royal Veterinary College for photographing the microscopic preparations. I am deeply grateful to Lt. Col. J. E. Smadel, of the U.S. Army Medical Corps, for the hospitality of his laboratory when he was in this country and for his invaluable advice and encouragement on many occasions.

REFERENCES

Andrewes, C. H., and Mills, K. C. (1943). *Lancet*, **1**, 292.
Coles, J. D. W. A. (1940). *Ondestepoort, J. vet. Sc. anim. Husb.*, **15**, 141.
Haagen, E., and Mauer, G. (1938), *Zbl. Bakt. (Abt. 1) Orig*, **143**, 81.
Meyer, K. F., Eddie, B., and Yanamura, H. (1942). *Proc. Soc. exp. Biol, N.Y.*, **49**, 609.
Meyer, K. F. (1944), in Biester and Devries *Disease of Poultry*. The Collegiate Press Inc. Ames. Iowa. U.S.A.
Pinkerton, H., and Swank, R. L. (1940). *Proc. Soc. exp. Biol, N.Y.*, **45**, 704.
Rasmussen, R. K. (1938). *Ugeskr. Laeg. Aargang*, **100**, 989. (Abstract and translation provided by Mr. James Fisher, of Oxford.)
Smadel, J. E., Wall, M. J., and Gregg, A. (1943). *J. exp. Med.*, **78**, 189.
Smadel, J. E., Jackson, E. B., and Harman, J. W. (1945). *Ibid.*, **81**, 385.
Smadel, J. E., Westman, K., and Reagan, R. L. (1943). *Proc. Soc. exp. Biol, N.Y.*, **54**, 70.

[*Received for publication October 17th, 1946.*]

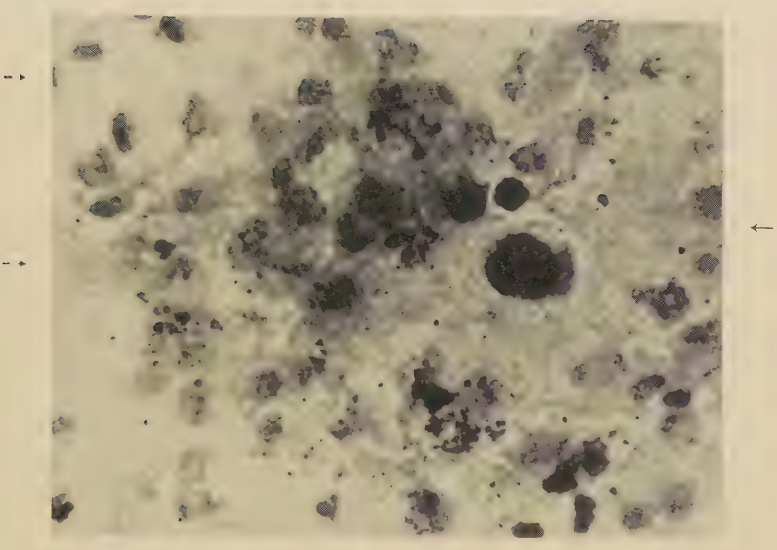


FIG. 1.

Elementary bodies of Ornithosis Virus in pericardial exudate of a pigeon.

Note the wide distribution of elementary bodies throughout the field and one dense intra-cytoplasmic collection and two looser aggregations. × 1100.

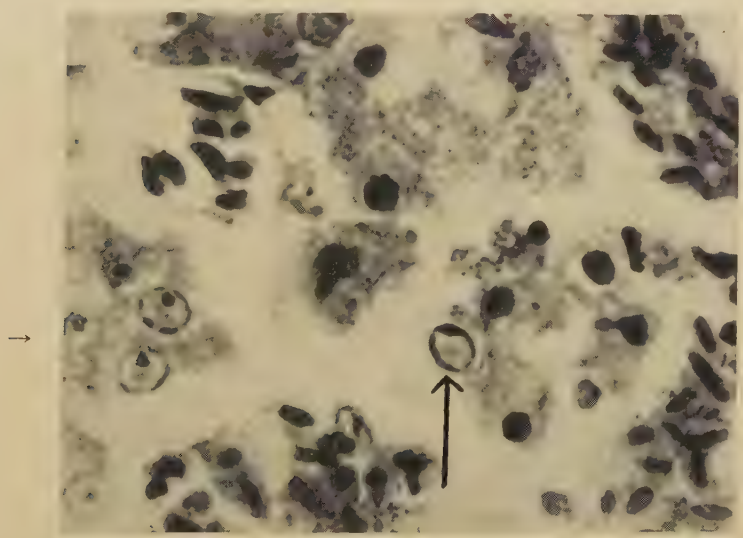


FIG. 2.

Liver cells in a pigeon showing intranuclear inclusions. × 1100.

SALMONELLA TYPHI MURIUM IN PENGUINS

By

T. A. Cockburn,

Zoological Society of London

Salmonella typhi murium is a well-known pathogen among animals such as the mouse, guinea-pig, and poultry, but the writer knows of no record of its occurrence in penguins. In June, 1946, the organism was isolated from pus in an airsac of a penguin which died of *Aspergillus fumigatus* infection. At the time there was a collection of 39 penguins, (*Spheniscus demersus*) in the Zoological Gardens, divided into three groups—seven in the Sanatorium Yard and 16 each at the Mappin Terrace and Penguin Pools. The bird in which the organism was found came from the Sanatorium Yard.

Three weeks later, three more penguins from the Yard died suddenly and at *post-mortem* examination no definite cause of death was discovered. Samples of heart blood from two of the penguins showed a double infection in each case with *Salmonella typhi murium* and *Salmonella newport*. The remaining three penguins were isolated for two weeks, but as they appeared to be normal two were sent to Mappin Terrace Pool and the third to the other pool. Four days later, nine of the Mappin Terrace and seven of the Penguin Pool birds were seriously ill and at the time of discovery four were moribund. The affected birds were listless with ruffled feathers and glazed eyes: two had green diarrhoea. The weight of a penguin is 6 to 8 lb. All were given 20,000 units of penicillin immediately and 0·5 g. sulphamezathine twice daily by mouth, but eight birds died within a few hours. At *post-mortem* examination nothing abnormal could be found, except that in two cases the rectums were moderately inflamed. *Salmonella typhi murium* was isolated in three out of the six heart-bloods cultured. The organism isolated previously had been found to be 1-12th to 1-20th as sensitive as *Bact. dys. flexneri* to sulphathiazole. It is possible, therefore, that organisms would have been obtained from some of the negative specimens but for the treatment.

On the day of the epidemic, the pools had had their routine three daily changes of water and washing down with strong hypochlorous acid solution. All penguins were isolated, each in a separate wooden box, and samples of each individual's droppings cultured for pathogenic organisms. Only two birds, Nos. 183 and 109, gave positive cultures of known pathogens, both having *Salmonella typhi murium* and these were kept for further examination. All remaining birds were returned to their pools. Penguins 183 and 109 were two of the three survivors of the Sanatorium Yard epidemic, and had been sent to the Mappin and Penguin Pools respectively only four days before the outbreaks there.

No. 183 was treated with sulphaguanidine, 0·5 g. twice daily, for ten days, and three days after commencement of treatment gave

a negative dropping. After six such negative findings at three-day intervals, the bird was returned to the remainder of the collection in the Penguin Pool. No. 109 was examined weekly for a month and on each occasion *S. typhi murium* was found in its droppings. Having thus been proved a carrier it was then given the same treatment as 183, with a similar result.

The isolation of an organism in the heart-blood of a bird does not prove that the death was due to such an infection, especially when the bird has been dead a number of hours before the blood was examined. Nevertheless the fact that no other cause of death could be found and the way in which the epidemic occurred in both pools four days after the introduction of a carrier of *S. typhi murium* to each is strong presumptive evidence.

SUMMARY

An outbreak of apparently infectious disease among penguins is described.

Salmonella typhi murium was isolated from some of the penguins. Two penguins were found to be healthy carriers of the organism.

The carriers were successfully treated by large doses of "sulpha" drugs. Penguins can tolerate up to 1 g. per day of some of these drugs with no apparent adverse effect.

ACKNOWLEDGMENTS

I wish to thank Dr. Joan Taylor of the Salmonella Reference Laboratory and Dr. Tomlinson of the Central Public Health Laboratory, Colindale, for their help in the bacteriological investigations.

[*Received for publication October 23rd, 1946*]

The Power to Destroy

Less dramatic . . . but no less destructive than the deadliest weapons of war . . . are the common pathogenic bacteria. Fortunately, Medicine has in Tincture Metaphen an agent of defence whose potency has been repeatedly—and impressively—demonstrated. Two investigators recently tested Tincture Metaphen against fifteen other antiseptic agents.* They found that, on the oral mucosa, Tincture Metaphen reduced bacteria count 95 to 100 per cent within five minutes; had a duration of action of two hours—about 200% longer than for any other antiseptic tested; and caused only a slight irritation in some cases, none in the others. Tincture Metaphen is supplied in 1, 4, 16 and 80 fluid ounce bottles. Abbott Laboratories (England) Limited, Wadsworth Road, Perivale, Middlesex.

** Meyer, E., and Arnold, L., Amer. J. Digest. Dis., 5:418*

Abbott

TINCTURE METAPHEN

(Tincture of 4-nitro-anhydro-hydroxy-mercury-orthocresol, Abbott)

W. Watson & Sons, Ltd.

Established 1837

313, HIGH HOLBORN, LONDON, W.C.1.

Manufacturers of

MICROSCOPES AND ALL ALLIED APPARATUS

PHOTOGRAPHIC LENSES, CAMERAS, ETC.

OPTICAL ELEMENTS for all purposes

Watson's output is controlled at present, many new and interesting designs are being developed. Send your enquiries for your requirements, an indication of delivery date and price will be quoted.

Telegrams: Optics, Holb. London

'M&B 693' Sulphapyridine

TRADE MARK BRAND

From the period of the early trials of 'M & B 693' in veterinary practice to the present day there has been a marked increase in the use of this chemotherapeutic agent and it is now established as the leading derivative of sulphanilamide employed by the veterinary profession.

It is indicated in streptococcal, coliform and many other bacterial infections, and prominent under this heading are infectious pneumonia of calves, pneumonia in adult cattle, joint ill, white scour in calves and pig pneumonia and paratyphoid.

	s.	d.
Containers of 100 × 0·125 gramme tablets	4	0
50 × 0·50 ,, ,,	3	4
250 × 0·50 ,, ,,	15	0
1000 × 0·50 ,, ,,	56	8
Containers of 25 grammes powder ...	3	3
500 ,, ,, ...	56	4

VETERINARY PRODUCTS M&B TO THE PROFESSION ONLY

Special quantity contract rates will be quoted on request.

MANUFACTURED BY

MAY & BAKER LTD.

DISTRIBUTORS

PHARMACEUTICAL SPECIALITIES (MAY & BAKER) LTD., DAGENHAM

9045

MEDICAL BOOKS

Telephone
EUSton 4282
(5 lines)

BOOKSELLING DEPARTMENT

Large stock of Textbooks and recent literature in all branches of Medicine and Allied Sciences.

SECONDHAND DEPARTMENT, 140, GOWER STREET, W.C.1

Large stock of secondhand recent editions always available.

LENDING LIBRARY (Medical and Scientific)

ANNUAL SUBSCRIPTION, Town or Country, from ONE GUINEA. All the latest works on Medicine and Allied Sciences obtainable. Bi-monthly list of New Books and New Editions added to the Library, post free to any address regularly.

THE LIBRARY CATALOGUE revised to December, 1943, containing classified index of authors and subjects. To subscribers 12s. 6d. net. To non-subscribers 25s. net., postage 8d.

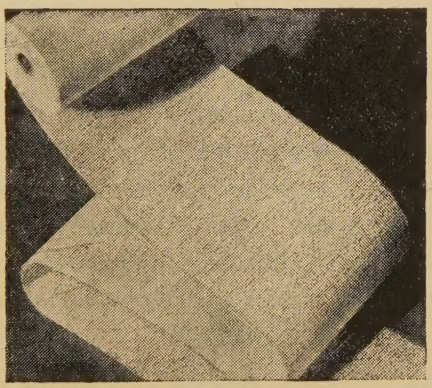
Supplement 1944 to December 1946 in preparation.

Details of terms and Prospectus free on request

Telegrams: "Publicavit, Westcent, London."

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET, W.C.1



The even impregnation and perfect finish of the 'Gypsona' Plaster of Paris Bandage ensure a light cast of great strength.

Gypsona
TRADE MARK

PLASTER OF PARIS BANDAGES, WIDE MATERIAL AND SLABS

Made in England by
T. J. Smith & Nephew Ltd., Hull.

'WELLCOME' BRAND BIOLOGICAL PREPARATIONS

for veterinary use

'Wellcome' Brand Veterinary Biological Preparations are produced by Laboratories which have made many important contributions to veterinary medicine. Systematic research has developed these preparations to a high level of efficiency and reliability, and is continuously devoted to the investigation of new applications of serotherapy to the control of animal disease. A critical and discriminating policy ensures that no such preparation is offered to the veterinary profession without adequate evidence of its therapeutic or prophylactic value.

LAMB DYSENTERY · BRAXY · TETANUS · STRIKE
C. PYOGENES INFECTIONS · BLACKLEG PULPY KIDNEY
DISEASE · CANINE BRONCHISEPTICUS INFECTION
CANINE DISTEMPER · STAPHYLOCOCCAL INFECTIONS
BOVINE EPIZOOTIC ABORTION · SWINE ERYSIPELAS

Prepared at
THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
SUPPLIED BY
BURROUGHS WELLCOME & CO.
(THE WELLCOME FOUNDATION LTD.)
12, RED LION SQUARE, LONDON, W.C.1.
(Temporary address)
CHAncery 6621

'TETMOSOL'

brand

TETRAETHYLTHIURAM MONOSULPHIDE SOLUTION (25%)

For the treatment of Mange

Comprehensive clinical trials conducted by a large number of veterinarians during the last few years have shown that sarcoptic and allied types of mange in animals are readily susceptible to 'Tetmosol' Alcoholic Solution (25%).

'Tetmosol' combines the advantages of high acaricidal activity, freedom from toxicity, cleanliness in use, and absence of objectionable odour.

For most cases two or three applications at intervals of a few days are all that are required and since 'Tetmosol' Solution is non-greasy and does not stain, small animals under treatment may be allowed to remain at home.

'Tetmosol' Alcoholic Solution (25%) is available in bottles of 100 c.c., 250 c.c. and 2 litres.

Literature will be forwarded on request.

IMPERIAL CHEMICAL [PHARMACEUTICALS] LTD.
THE RIDGE, BEECHFIELD ROAD, ALDERLEY EDGE, MANCHESTER

Ph.1 64b